AD-A260 226

AD ________________ (2)

EVALUATION OF SEVERAL BIOLOGICAL MONITORING TECHNIQUES FOR HAZARD ASSESSMENT OF POTENTIALLY CONTAMINATED WASTEWATER AND GROUNDWATER

VOLUME 2 - ABERDEEN PROVING GROUND WASTEWATER TREATMENT PLANT

FINAL REPORT

Dennis T. Burton, Ph.D.
Randall S. Herriott, B.A.
University of Maryland System
Wye Research and Education Center
P.O. Box 169, Queenstown, MD 21658

July 1992

Supported by

U. S. Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21702-5012

U. S. Navy Contract No. N00039-89-C-0001
The Johns Hopkins University
Applied Physics Laboratory
Laurel, MD 20723-6099

Project Officer: Tommy R. Shedd, M.S.
U. S. Army Biomedical Research and
Development Laboratory
Fort Detrick, Frederick, MD 21702-5010

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents

NOTICE

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Disposition

Destroy this report when it is no longer needed. Do not return it to the originator.

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): WREC-92-B5	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of Maryland System; 6b. OFFICE SYMBOL (If applicable): MINAR	7a. NAME OF MONITORING ORGANIZATION: U.S. Army Biomedical Research and Development Laboratory
6c. ADDRESS (City, State, and ZIP Code): Wye Research and Education Center, P.O. Box 169, Queenstown, MD 21658	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21702-5010
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research and Development Center; 8b. OFFICE SYMBOL (If applicable): USAMRDC	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: U.S. Navy Contract N00039-89-C-0001
8c. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21702-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification) Evaluation of Several Biological Monitoring Techniques for Hazard Assessment of Potentially Contaminated Wastewater and Groundwater. Volume 2 — Aberdeen Proving Ground Wastewater Treatment Plant.

12. PERSONAL AUTHOR(S) Burton, Dennis T. and Randall S. Herriott

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 11/88 TO 12/91	July 1992	132

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Wastewater, aquatic, acute toxicity, chronic toxicity, mutagenicity, Ames, teratogencity, FETAX, carcinogenicity, ventilatory biomonitoring system, Microtox[R], Photobacterium

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

An evaluation of several biological monitoring techniques for hazard assessment of potentially contaminated effluent was conducted at the Aberdeen Proving Ground Wastewater Treatment Plant (APG-WWTP), Aberdeen Proving Ground, MD, from early May 1990 to February 13, 1991. An array of biomonitoring tests structured in a tiered hazard assessment framework was used in the evaluation of the effluent. Several levels of biological organization were included in the array of tests.

Acute toxicity was evaluated on daily 24-h composite samples using a 5- and 15-min Microtox[R] assay which employs microbial (Photobacterium phosphoreum) bioluminescent activity. Three 24-h LC50 rotifer (Brachionus rubens) toxicity tests were conducted using 24-h composite samples. The following chronic tests were all performed three times using 24-h composite samples: 96-h EC50 algal (Selenastrum capricornutum) growth test, 7-d daphnid (Ceriodaphnia dubia) survival and reproduction test, and 7-d fathead minnow (Pimephales promelas) survival

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary F. Bostian	22b. TELEPHONE (Include Area Code): 301-619-7326; 22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE: Unclassified

18. SUBJECT TERMS (Continued)

phosphoreum, Rotifer, *Brachionus rubens*, green alga, *Selenastrum capricornutum*, daphnid, *Ceriodaphnia dubia*, fathead minnow, *Pimephales promelas*, African clawed frog, *Xenopus laevis*, Japanese medaka, *Oryzias latipes*, bluegill, *Lepomis macrochirus*.

19. ABSTRACT

and growth test. The acute rotifer tests and all chronic tests were conducted during the same periods in order to compare toxicological responses between biomonitoring systems.

Mutagenicity assays (Ames) were performed three times on both the effluent and diluent water using 24-h composite samples. Two preliminary 96-h (flow-through) teratogenicity tests were conducted using the African clawed frog (*Xenopus laevis*) embryo teratogenesis assay (FETAX). A 6-month carcinogenicity test was conducted under flow-through test conditions with Japanese medaka (*Oryzias latipes*) unexposed fry and fry initiated with diethylnitrosoamine. The U.S. Army Biomedical Research and Development Laboratory's (USABRDL) 21-d bluegill (*Lepomis macrochirus*) computerized ventilatory monitoring system, which has been designed to detect unexpected abrupt changes in water quality or episodic events, was tested two times. Comprehensive chemical analyses were performed four times on 24-h composite samples of both the effluent and diluent water. Routine water quality was also determined frequently throughout the 6-month carcinogenicity study.

EXECUTIVE SUMMARY

An evaluation of several biological monitoring techniques for hazard assessment of potentially contaminated effluent was conducted at the Aberdeen Proving Ground Wastewater Treatment Plant (APG-WWTP), Aberdeen Proving Ground, MD, from early May 1990 to February 13, 1991. An array of biomonitoring tests structured in a tiered hazard assessment framework was used in the evaluation of the effluent. Several levels of biological organization were included in the array of tests.

Acute toxicity was evaluated on daily 24-h composite samples using a 5- and 15-min Microtox® assay which employs microbial (*Photobacterium phosphoreum*) bioluminescent activity. Three 24-h LC50 rotifer (*Brachionus rubens*) toxicity tests were conducted using 24-h composite samples. The following chronic tests were all performed three times using 24-h composite samples: 96-h EC50 algal (*Selenastrum capricornutum*) growth test, 7-d daphnid (*Ceriodaphnia dubia*) survival and reproduction test, and 7-d fathead minnow (*Pimephales promelas*) survival and growth test. The acute rotifer tests and all chronic tests were conducted during the same periods in order to compare toxicological responses between biomonitoring systems.

Mutagenicity assays (Ames) were performed three times on both the effluent and diluent water using 24-h composite samples. Two preliminary 96-h (flow-through) teratogenicity tests were conducted using the African clawed frog (*Xenopus laevis*) embryo teratogenesis assay (FETAX). A 6-month carcinogenicity test was conducted under flow-through test conditions with Japanese medaka (*Oryzias latipes*) unexposed fry and fry initiated with diethylnitrosoamine. The U.S. Army Biomedical Research and Development Laboratory's (USABRDL) 21-d bluegill (*Lepomis macrochirus*) computerized ventilatory monitoring system, which has been designed to detect unexpected abrupt changes in water quality or episodic events, was tested two times. Comprehensive chemical analyses were performed four times on 24-h composite samples of both the effluent and diluent water. Routine water quality was also determined frequently throughout the 6-month carcinogenicity study.

The array of biological monitoring techniques used to assess the potential toxicity of the APG-WWTP effluent showed that the effluent generally was not toxic during most of the study period. Toxicity was detected by the following test systems. Acute toxicity was found in ≈10% of the effluent samples measured (16 of 156 samples) via Microtox®. Toxicity appeared to occur in a random pattern over the 6-month test period. The cause of the acute toxicity measured by Microtox® was not obvious. No acute toxicity was found in the 24-h rotifer tests.

Chronic toxicity was detected during the February 1991 series of tests by two out of the three biomonitoring systems used. A significant reduction in growth occurred in the algal growth test at 100% effluent; no toxicity occurred at lower concentrations. Significant mortality occurred to larval fathead minnow exposed to APG-WWTP effluent at concentrations above 6.25% effluent by volume. A comprehensive chemical analysis conducted on a 24-h composite sample taken during the February 1991 period when chronic toxicity occurred showed that several EPA priority pollutant organics were present in the effluent which were not present in three prior effluent samples. However, the concentration of each priority pollutant was substantially below EPA water quality criteria concentrations for the compounds. No chronic toxicity was found using the algal, daphnid, or fathead minnow tests during the July 1990 and November 1990 test periods.

No mutagenicity was detected in unconcentrated APG-WWTP effluent, unconcentrated dechlorinated APG diluent water or concentrated (10X) APG diluent water. Mutagenicity was found in the November 1990 and February 1991 effluent samples which were concentrated 10X; no mutagenicity was observed in the September 1990 concentrated (10X) effluent sample. No teratogenicity data are available because only preliminary tests were conducted.

The following carcinogenicity events were found in the study. Liver neoplasms and foci of cellular alteration occurred at a slightly higher incidence in fish in the groups exposed to APG-WWTP effluent than in APG diluent water controls at the interim (day 121 of exposure), chronic (day 200 of exposure), and recovery observation periods. Incidence of lesions were similar in control fish held for the same length of time in USABRDL well water at Ft. Detrick, Frederick, MD. Hepatic vacuolation and cystic degeneration occurred in several fish in most groups at all three observation periods; a slight increase in the severity of these lesions was observed in the fish exposed to effluent at the 121- and 200-d observation periods.

Changes in kidney and thyroid tissue occurred in fish at each observation period of the study and in all groups; however, no apparent pattern of incidence was present which could be related to exposure. Nonhepatic neoplasms which occurred infrequently, but only in fish exposed to APG-WWTP effluent, included lymphosarcoma, mesothelioma, ocular medulloepithelioma, ovarian teratoma, gas gland epithelioma, and thyroid follicular cell neoplasms.

No abrupt changes in effluent quality or episodic events occurred during the two biological monitoring early warning system tests. The effluent was not overtly toxic to the bluegills during the 14-d definitive phases of each test. That is, significant mortality did not occur during 14 d of exposure to 100% effluent.

ACKNOWLEDGEMENTS

We thank Mr. Tommy R. Shedd, Mr. Henry S. Gardner, and Dr. Robert A. Finch of the Research Methods Branch/Health Effects Research Division of the U.S. Army Biomedical Research and Development Laboratory (USABRDL), Fort Detrick, Frederick, Maryland, for their continual support and help throughout the study. We thank Dr. William H. van der Schalie, currently at the U.S. Environmental Protection Agency, Washington, DC, for initiating the study. We acknowledge the Research Methods Branch/Health Effects Research Division of the U.S. Army Biomedical Research and Development Laboratory for supporting the study via Navy Contract No. N00039-89-C-0001 to the Johns Hopkins University Applied Physics Laboratory. We would also like to thank the University of Maryland System, Maryland Institute for Agriculture and Natural Resources, Agricultural Experiment Station, Wye Research and Education Center for partial support during the writing of the report.

TABLE OF CONTENTS

LIST OF TABLES

SECTION 1

INTRODUCTION

The Johns Hopkins University/Applied Physics Laboratory-Aquatic Ecology Section (JHU/APL-AES) under contract to the Health Effects Research Division of the United States Army Biomedical Research and Development Laboratory (USABRDL) conducted an on-site study from early May 1990 to February 13, 1991, to determine the use of several biological monitoring techniques for hazard assessment of potentially contaminated effluent at the Aberdeen Proving Ground Wastewater Treatment Plant (APG-WWTP), Aberdeen Proving Ground, MD. The first three months of the study were used for facility modification, set-up, and range finding tests. The definitive experimental phase of the study was conducted over a 6-month period from August 22, 1990 to February 13, 1991.

APG-WWTP effluent (NPDES Permit No. MD 0021237; Outfall 001) used in the study was the final tertiary treated product of a raw influent which included a variable combination of domestic, munitions, and industrial sources. The plant has a designed capacity of 2.8 mgd; however, the actual capacity was 1.5 mgd with an average of 1.0 mgd (Logan, 1992). Chlorination was used for disinfection followed by dechlorination (sulfur dioxide) of the effluent before discharge.

An array of biomonitoring tests structured in a tiered hazard assessment framework was used in the evaluation of the effluent. Several levels of biological organization were included in the array of tests. The effluent was tested for acute and chronic toxicity; mutagenic, teratogenic, and carcinogenic potential; and chemical composition. In addition, USABRDL's biological monitoring early warning system was tested.

SECTION 2

OBJECTIVES OF STUDY

1) To evaluate acute toxicity of the effluent using the 5- and 15-min Microtox® procedure (Photobacterium phosphoreum bioluminescent activity) and the 24-h LC50 Rotifer Toxkit™ (Brachionus rubens) screening test.

2) To evaluate chronic toxicity using the 96-h EC50 algal (Selenastrum capricornutum) growth test, 7-d daphnid (Ceriodaphnia dubia) survival and reproduction test, and 7-d fathead minnow (Pimephales promelas) survival and growth test.

3) To determine the mutagenic potential of unconcentrated and concentrated (10X) samples of the effluent using the Ames assay.

4) To determine teratogenic potential of the effluent using the frog (Xenopus laevis) embryo teratogenesis assay - Xenopus (FETAX).

5) To determine carcinogenic potential of the effluent using a a 6-month Japanese medaka (Oryzias latipes) test.

6) To test USABRDL's 21-day bluegill (Lepomis macrochirus) biological monitoring early warning system which can detect rapid changes in the acute toxicity of the effluent.

7) To quantify the major chemicals present in the effluent and monitor the general water quality of the effluent.

SECTION 3

MATERIALS AND METHODS

3.1 Background Information

The study was conducted on-site in USABRDL's Aquatic Biomonitoring Trailer Version 1.0. A complete description of the trailer layout, associated equipment and instrumentation, study protocols, etc., may be found in Herriott and Burton (1992). Briefly, the biomonitoring trailer is a specially designed 8 ft x 24 ft mobile laboratory which is divided into two compartments: a small room (8 ft x 5 ft) used primarily to isolate fish used in the ventilatory biological monitoring system and a two-tiered large room (8 ft x 19 ft) used for flow-through toxicity testing (e.g., teratogenicity and carcinogenicity) water quality testing, storage of test materials, and data acquisition. The trailer is supplied with a 240 volt (single phase), 100 amp power supply and a back-up generator.

APG-WWTP provided additional space in the plant's pump house for a water filtration system, aeration/equilibration tanks, water sampler, water pumps, air compressor, and bluegill acclimation space. Aberdeen dechlorinated potable water (charcoal filtered) which was used as diluent water and APG-WWTP effluent were supplied to the trailer via PVC pipe. Excess diluent water and effluent from the trailer were collected and returned to the plant for further treatment before being discharged.

Acute toxicity was evaluated daily on 24-h composite samples using the 5- and 15-min Microtox® assay which employed microbial (Photobacterium phosphoreum) bioluminescent activity. Three 24-h LC50 rotifer (Brachionus rubens) toxicity tests were conducted using 24-h composite samples. The following chronic tests were all performed three times using 24-h composite samples as described below: 96-h EC50 algal (Selenastrum capricornutum) growth test, 7-d daphnid (Ceriodaphnia dubia) survival and reproduction test, and 7-d fathead minnow (Pimephales promelas) survival and growth test. The acute rotifer tests and all chronic tests were conducted during the same periods in order to compare toxicological responses between test systems. A summary of the sample periods for all tests is given in Table 1.

Mutagenicity assays (Ames) were performed three times on both the effluent and diluent water using 24-h composite samples. Two preliminary 96-h (flow-through) teratogenicity tests were conducted using the African clawed frog (Xenopus laevis) embryo teratogenesis assay (FETAX). A 6-month carcinogenicity test was conducted under flow-through test conditions with Japanese medaka (Oryzias latipes) unexposed fry and fry initiated with

diethylnitrosoamine (DEN). USABRDL's 21-d bluegill (Lepomis macrochirus) computerized ventilatory monitoring system was tested two times. Comprehensive chemical analyses were performed four times on 24-h composite samples of both the effluent and diluent water. Routine water quality was also determined frequently throughout the 6-month carcinogenicity study.

3.2 Acute Toxicity

3.2.1 Microtox® Test

The Microtox® test (Microbics Corp., Carlsbad, CA) is a rapid acute toxicity test that may be completed in less than one hour. The test is based on the reduction in bioluminescence of the marine bacterium P. phosphoreum when exposed to a sample of unknown toxicity. The degree of light reduction, an indication of metabolic inhibition in the test organisms, indicates the degree of toxicity of the sample. The Microtox® test procedures followed were those outlined in Herriott and Burton (1992) which were derived from Microtox®'s operating manual (Microtox®, 1988). A Microtox® Model 500 Analyzer with PC version 5.20 software was used for both a 5-min and 15-min test on all samples.

Microtox® tests were conducted from September 5, 1990 until the termination of the carcinogenicity study on February 13, 1991. Composite samples (24 h) of 100% effluent were collected daily by an Isco® refrigerated sampler (Model 2700R; Isco Inc., Lincoln, NE). One liter aliquots of the 24-h composite effluent samples were siphoned into 1 L Nalgene polycarbonate bottles and held at 4°C. Microtox® tests were conducted on-site three times a week (Monday, Thursday, and Friday). Samples collected on Saturday, Sunday, and Monday were analyzed on Monday and samples collected on Tuesday, Wednesday, and Thursday, were analyzed on Thursday. The Friday sample was analyzed on Friday.

3.2.2 Rotifer Toxicity Test

The potential toxicity of the effluent was determined three times using the Rotifer Toxkit™ Screening Test (US TOXKIT, Tampa, FL). The test utilized newly hatched rotifers (B. rubens) <4 h old. The rotifers used in the tests were hatched from cysts supplied in the Rotifer ToxKit™. Rotifer ToxKit™ synthetic medium was used to hatch the cysts and rear the organisms before testing. The static tests were conducted in glass Petri dishes containing 10 mL of test solution. All rotifer tests were conducted at The Johns Hopkins University Applied Physics Laboratory-Aquatic Ecology Section (JHU/APL-AES) Laboratory in Shady Side, MD.

Preliminary tests showed that 100% effluent was not toxic; therefore, 100% APG-WWTP effluent only was tested in two of the three tests. A fourth test concentration of 12.5% effluent was

tested during the last test conducted in February 1991. ToxKit™ synthetic medium and APG diluent water were run as controls. The effluent used in each test was taken from a 24-h composite sample which was collected in a refrigerated Isco® sampler (Model 2700R; Isco Co., Lincoln, NE). The effluent, which was used within 24 h from the time of collection, was held in glass containers at 4°C until used in the tests. Three replicates of 10 organisms each were performed at each test concentration. All tests were conducted at 25 ± 0.5°C. Routine water quality (alkalinity, conductivity, DO, hardness, pH, and temperature) was taken at the beginning and end of each test. All tests were conducted under a 16-h light:8-h dark photoperiod (fluorescent lights at 60-85 foot candles).

3.3 Chronic Toxicity

All chronic tests were conducted at the JHU/APL-AES Laboratory using non-chlorinated deep well water as diluent water. A comprehensive chemical analysis of the JHU/APL-AES diluent water is given in Table 2. The effluent samples used in all tests were taken from 24-h composite samples which were collected in a refrigerated Isco® sampler (Model 2700R; Isco Co., Lincoln, NE). All effluent was transported to the laboratory in glass containers placed on ice and held at 4°C until used in the tests. One 24-h composite sample was used for each algal test within 24 h of collection. Three 24-h composite samples, which were collected, transported, and held as described above, were obtained on days 1, 3, and 5 of the 7-d tests with both the invertebrate and fish. Both the daphnid and fathead minnow tests were conducted using aliquots taken from the same effluent sample.

3.3.1 Green Algal Growth Test

A S. capricornutum starter culture was obtained from the culture collection at North Texas State University, Denton, TX. Stock algal cultures were reared in 2.5 L Pyrex culture flasks containing 1 L of sterilized double strength "AAP" algal assay medium, with sufficient P added to achieve a 20:1 N:P ratio as described in Miller et al. (1978). Cultures were maintained in a constant temperature incubator under constant cool-white fluorescent lights (≈300 foot candles) at a temperature of 20 ± 1°C on a shaker table oscillating at 100 rpm (± 10 %). Log growth cells were used to start all tests.

The potential toxicity (96-h EC50 for growth) of the effluent to S. capricornutum was determined three times (Table 1) by the procedures given in Horning and Weber (1985). The nutrient media used for the bioassays was sterilized double strength "AAP" algal assay medium, with sufficient P added to achieve a 20:1 N:P ratio as described in Miller et al. (1978) rather than the media recommended in the test method.

Algal test solutions were prepared by dilution of the effluent with filtered sterilized assay media within a sterile transfer room. Test solutions (100 mL total volume) were dispensed into 250 mL Delong flasks and inoculated with S. capricornutum cells in log growth to achieve a density of ≈5 x 10^3 cell/mL. Triplicates were prepared for each treatment. The flasks were placed on a shaker table in an incubator set at the culturing conditions described above. Growth measurements (cell density) were made from all replicates in each treatment at 0, 24, 48, 72, and 96 h. Algal cell density was determined from a 1 mL sample with a Model ZBI Coulter Counter (Coulter Electronics Inc., Hialeah, FL). The instrument was calibrated with each use via hemocytometer counts.

3.3.2 Daphnid Survival and Reproduction Test

The cladoceran, C. dubia, was cultured at 25 ± 1°C in 600 mL glass beakers filled with 400 mL JHU/APL-AES well water amended with selenium (2 ug Se/L as Na_2SeO_3) as recommended by Winner (1987 and 1989). The diet consisted of a mixture of Cerophyl® (Cerophyl Laboratories, Inc., Kansas City, MO) and the green alga, S. capricornutum, added to the daphnid culture to achieve final concentrations of 120 ug Cerophyl®/mL and 6.7 x 10^5 S. capricornutum cells/mL. Starter cultures of C. dubia were obtained from the Center for Lake Superior Environmental Studies, University of Wisconsin - Superior.

The chronic toxicity of the effluent to Ceriodaphnia was determined three times (Table 1) by the method given in Draft No. 3 of the ASTM proposed guide for conducting three brood, renewal toxicity tests (Waller and Lazorchak, 1986). All neonates used in the 7-d survival and reproduction tests were produced by daphnids in culture that had released at least three broods. The initial age of the neonates in each test was <24 h old. The tests were conducted in 50 mL glass beakers containing 30 mL of test solution. All tests were conducted in an environmental chamber at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles at the surface of the culture vessels). All test organisms were fed daily as described above at each 24-h renewal. Routine water chemistry was taken at each renewal.

3.3.3 Fathead Minnow Survival and Growth Test

Fathead minnow (P. promelas) larvae, <24 h at the start of the tests, were obtained from the JHU/APL-AES culture maintained at 25 ± 1°C in JHU/APL-AES well water. The JHU/APL-AES culture procedures were similar to those recommended by Peltier and Weber (1985). The JHU/APL-AES culture was initiated with mature fathead minnows obtained from the U.S. EPA Environmental Monitoring and Support Laboratory - Cincinnati, Ohio. Briefly, spawning fish were cultured in fiberglass tanks (2.4 x 0.8 x 0.5 m)

containing 0.2 m JHU/APL-AES well water held at 25 ± 1°C. The spawning adults were fed a diet of frozen brine shrimp (Artemia sp.; Argent Chem. Lab., Redmond, WA) and TetraMin® Staple Food (Ramfab Aquarium Products Co., Oak Ridge, TN) twice daily. Excess food was removed daily. Five sets of spawning fathead minnows were maintained in the culture tanks at a ratio of 1 male:3 females. Replacement spawners were rotated at approximately 3-month intervals. Fathead minnow embryos were collected on spawning substrates (10 cm I.D. x 20 cm long PVC pipe sections cut longitudinally in equal portions) and transferred to 19 L aquaria at 25 ± 1°C in JHU/APL-AES well water for hatching. All stages of the fish were reared under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles).

The chronic toxicity of the effluent to fathead minnows was determined three times (Table 1) by the static renewal method (solutions renewed daily) given in Weber et al. (1989). All larvae used in the 7-d survival and growth tests were <24 h old. The tests were conducted in 600 mL glass beakers containing 500 mL of test solution. All test organisms were fed brine shrimp (Artemia sp.) nauplii <24 h old daily at each 24-h renewal. All tests were conducted at 25 ± 1°C under a 16-h light:8-h dark photoperiod (fluorescent lights; 60-85 foot candles). Routine water chemistry was taken at each renewal. Dry weight was determined by drying at 100°C for a minimum of 12 h.

3.4 Mutagenicity

Salmonella/mammalian-microsome reverse mutation assays (Ames test) were conducted three times (Table 1) on APG-WWTP effluent and APG diluent water samples. Ames assays were conducted on both unconcentrated and concentrated (10X via XAD-2 resin extracts) samples of the effluent and diluent water. The Ames mutagenicity assays were conducted by Hazleton Laboratories America, Inc., Kensington, MD.

Composite samples (24 h) of effluent were collected in 45-L (12 gallon) polypropylene containers packed in ice by Isco™ samplers (Model 2700; Isco Inc., Lincoln, NE). Grab samples of diluent water were collected in a large polypropylene tank with a 99% particle replacement time of ≈12 h. Thirty-one liters (1 L for the unconcentrated sample and 30 L for the 10X sample) of each material were siphoned into appropriately labeled 1 L Nalgene polycarbonate bottles, packed in ice, and transported to Hazleton Laboratories America, Inc., in insulated containers. The unconcentrated samples were analyzed by Hazleton Laboratories America, Inc. Protocol No. HLA Protocol 401W, Edition 16. The concentrated (10X) samples collected on September 27, 1990 and November 7, 1990 were analyzed by Protocol No. HLA Protocol 401X, Edition 16. The sample collected on February 6, 1991 was analyzed by the procedures in Protocol No. HWA Protocol 401X,

Edition 17. Effluent and diluent water were also taken during the same sampling period for detailed chemical analyses (see Section 3.8.1).

The experimental procedures for the unconcentrated and 10X tests are given in the protocols shown above. Briefly, the mutagenicity assays evaluated the effluent and diluent water samples for their ability to induce reverse mutations at the histidine locus in the genome of specific S. typhimurium tester strains both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor 1254-induced rat liver. The tester strains used in the assays were TA98 and TA100. The assays were conducted using two plates per dose level in the presence of microsomal enzymes. Six dose levels of the effluent and diluent water samples were tested in both the presence and absence of S9 along with appropriate vehicle controls (three plates per dose), negative controls, and positive controls. Resin controls were also run for the 10X samples. The doses tested in the 10X assays varied based on the amount of extractable organics recovered from the test material.

3.5 Teratogenicity

Two preliminary teratogenicity tests (Table 1) were conducted using the frog embryo teratogenesis assay - Xenopus (FETAX) which is a 96-h quantitative teratogen assay used to screen for developmental toxicants in aquatic media. The preliminary FETAX assays were conducted under flow-through test conditions during the 6-month continuous exposure carcinogenicity test. Both assays were conducted by the method given in Draft No. 3 of the ASTM proposed guide for conducting FETAX (Bantle and Sabourin, 1990) with the following exception. The ASTM method states that five test concentrations plus controls should be used. However, only two flow-through effluent concentrations (100% effluent and 10% effluent by volume) plus controls were available in the mobile trailer because the FETAX tests were run in the same flow-through system used for the 6-month carcinogenicity test (see Section 3.6).

Embryos between normal stage 8 blastulae and normal stage 11 gastrulae were obtained from Xenopus breeding colonies at USABRDL. The embryos were suspended in FETAX solution in an Erlenmeyer flask and delivered to the trailer on the morning the test was initiated by USABRDL personnel. The embryos were de-jellied with 200 mL of a 2% L-cysteine solution (2 g of L-cysteine per 98 mL of FETAX solution). Once de-jellied, the embryos were rinsed and re-suspended in FETAX solution. The embryos were placed in twelve 250 mL mesh bottomed glass beakers (25 embryos/beaker) which were suspended by a wire harness (1 beaker per aquarium) in the 5 gallon aquaria used in the 6-month carcinogenicity test (4 aquaria at 100% effluent; 4 at 10%

effluent by volume; and 4 diluent water controls). The tests were conducted at 25 ± 1°C under a 16-h light: 8-h dark photoperiod (fluorescent lights; ≈75 foot candles).

The beakers were checked daily for mortality. At the end of the 96-h exposure, the organisms were anesthetized using MS-222 prior to formalin fixation. The test organisms were then placed in 20 mL scintillation vials containing a 3% formalin solution. All organisms were sent to USABRDL for morphological analysis by their FETAX staff.

3.6 Carcinogenicity

The Japanese medaka (O. latipes), which has been shown to be a sensitive laboratory carcinogen model (for ex., see Hawkins et al., 1988; Klaunig et al., 1984; Metcalfe, 1989), was used to screen for environmental pollutants which may induce neoplasms. Both unexposed and fry initiated with diethylnitrosoamine (DEN) were used in a 6-month continuous exposure test conducted in the mobile laboratory at the APG-WWTP from August 22, 1990 to February 13, 1991. The test was given the designation Carcinogenicity Test (T) by USABRDL.

Two test concentrations (100% effluent and 10% effluent by volume) plus APG diluent water (control) were used in the study. The test solutions were delivered by a solenoid-activated proportional dilutor system which was constructed primarily of glass and stainless steel; silicon tubing was also used. The test concentrations were delivered to twelve 19 L (5 gallon) aquaria (4 aquaria at 100% effluent; 4 at 10% effluent by volume; and 4 control aquaria); each aquarium contained a volume of ≈16 L (4.25 gallons). All aquaria were held in a constant temperature (25 ± 1°C) water bath. The dilutor was calibrated to complete one full cycle every 2.5-3.5 minutes. During a cycle, tanks 1-4 received 250 ± 50 mL of diluent water, tanks 5-8 received 250 ± 50 mL of 10% effluent by volume, and tanks 9-12 received 250 ± 50 mL of 100% effluent.

Both unexposed fry and fry (14-d old) exposed to DEN, were reared off-site at USABRDL until 25 days old. The fish were randomized into 6 groups of 60 fish/group for both the unexposed and DEN initiated organisms. The fish were suspended in twelve 1000 mL mesh-bottom glass beakers in the appropriate flow-through test aquaria in the mobile laboratory. The fish were held in the beakers for one week after which they were released into the aquaria.

Pre-adult fish, 25-30 days old, were fed Tetramin® flake food (2 feedings per day Monday, Wednesday, Friday, Saturday, and Sunday; and 1 feeding per day Tuesday and Thursday), live brine shrimp <48 h old (1 feeding per day, 40 brine shrimp per fish), and ground ocean plankton (Silco Pet Products Co., Alexandria,

VA) (1 feeding per day Tuesday and Thursday). Adult fish, 31 days or older, were fed Tetramin® flake food (2 feedings per day Monday through Friday and 1 feeding per day Saturday and Sunday), live brine shrimp (1 feeding per day Monday, Wednesday, and Friday), and ground ocean plankton (1 feeding per day Tuesday, Thursday, Saturday, and Sunday). Tanks were cleaned on an as needed basis (usually 1-2 times a week) by scrubbing algae from the sides of the tanks, allowing the debris to settle, and then siphoning. Tetramin® and ground ocean plankton were fed *ad libitum* for 15-30 minutes during each feeding.

The number of test organisms alive in each tank was monitored and recorded daily. Dead or moribund fish were fixed for subsequent pathological observation. The dilutor cycle time was calculated and recorded daily. The volume of effluent and diluent water delivered to the aquaria was checked weekly. When necessary, cycle time and/or volume distributions were adjusted. The dilutor was shutdown (for no more than one hour) and cleaned on an as needed basis. Daily water quality (DO, pH, and temperature) was determined in all aquaria. Additional water quality tests (alkalinity, hardness, chlorine, and ammonia-nitrogen) were performed twice a week in alternating aquaria (odd/even) throughout the study.

On day 121, all but 20 Japanese medaka in each tank were taken back to USABRDL for fixation and pathological observation. On day 200, when the exposure was completed, the remaining Japanese medaka were also taken back to USABRDL for recovery observations and subsequent pathological analysis.

3.7 Biological Monitoring Early Warning System

The 21-d bluegill (*L. macrochirus*) computerized ventilatory monitoring system, which is a real-time continuous monitoring system, was run in a field test mode to detect possible unexpected abrupt changes in effluent quality or episodic events which may be harmful to the aquatic environment. The system uses changes in fish ventilation frequency, opercular amplitude, and cough frequency to predict acute toxicological effects (Shedd et al., 1986). Individual fish in two control and two experimental groups of 8 fish/group (total of 32 fish) are held in the test system for a period of 21 days during a typical ventilatory test. The 21-d period includes an initial 3-d "acclimation" period (no data are collected during the 3-d period) followed by a 4-d period in which all 32 fish receive diluent water only in order to establish baseline data. At the end of the baseline period, two groups of 8 fish/group are switched to effluent for 14 d of monitoring while exposed to effluent. The fish are isolated from all activity including feeding during the 21-d period.

Two ventilatory tests were performed during the APG-WWTP study: Ventilatory Test I was conducted from July 21, 1991 -

August 10, 1990 and Ventilatory Test II from October 29 - November 19, 1990. Preliminary toxicity tests with the bluegill showed that the effluent was not acutely toxic; therefore, 100% effluent was used as the test concentration. APG-WWTP effluent and APG de-chlorinated diluent water were supplied to a four component ventilatory dilutor system which was calibrated to complete one full cycle every 55-65 seconds. During a cycle, 16 ventilatory chambers received 50 ± 2.5 mL of effluent, while the remaining 16 ventilatory chambers received 50 ± 2.5 mL of diluent water. A complete description of the ventilatory diluter system, ventilatory test chambers, components of the data acquisition system, etc., is given in Herriott and Burton (1992). Information concerning the software of the data acquisition system, acquisition of the automated water quality, etc. may be found in USABRDL (1991).

Juvenile bluegills (6.4-9.0 cm standard length; 2.5-3.5 inches) were reared off-site at USABRDL. Two weeks prior to each test, bluegills were delivered to the APG-WWTP study site for acclimation in APG diluent water. The fish were fed trout chow or frozen brine shrimp twice daily. Dead or moribund fish were removed and disposed of immediately to reduce the risk of disease to the other bluegills. Tanks were siphoned of debris daily and thoroughly cleaned once a week. The fish were held at 25 ± 2°C under continuous light (fluorescent lights; ≈75 foot candles).

On day 1 of the test, 32 bluegills were randomly transferred to 32 ventilatory chambers. Once placed in the ventilatory chambers, the fish were oriented to face the water input end of the test chamber. The ventilatory chambers were then connected to their designated leads to the biomonitoring data acquisition system. Signals from each test chamber were checked via an oscilloscope for clarity before initiating the test.

Computer and printer operation were checked daily. Entry into and exit from the biomonitoring trailer were recorded each time the event occurred. When entering and exiting the trailer, the computer screen was printed along with the entry or exit time. In addition, any unusual events (e.g., external noise, low DO, reduced water pressure) were noted during their occurrence. These data were collected to eliminate possible false events during a ventilatory run. The ventilatory signal of each fish was checked daily via an oscilloscope and the data acquisition system.

The cycle times of dilutors 1-4 were measured, calculated, and recorded daily. When necessary, cycle times were adjusted. The high and low electrodes located in each mixing chamber were inspected daily and cleaned on an as needed basis. Aeration was performed in the 100% effluent mixing chambers to increase DO concentrations. All solenoids and delivery lines were inspected daily to ensure that they were operating properly.

Routine water quality was measured via grab samples taken from a dilutor flow splitting cup containing 100% effluent and one containing diluent water as described in Section 3.8.2. Water quality was also monitored continuously and logged on the data acquisition system as described in Section 3.8.4.

At the end of the test all bluegills were weighed (wet weight) and measured (total length). The volume of effluent or diluent water delivered to each ventilatory chamber was measured and recorded. The data from each test were transferred from the data acquisition system to floppy disks for subsequent analysis at USABRDL.

3.8 Chemical Analyses

3.8.1 Comprehensive Chemical Analyses

Comprehensive chemical analyses were performed four times on 24-h composite samples of APG-WWTP effluent and APG dechlorinated tap water by Biospherics Inc. (Beltsville, MD) as shown in Table 1. APG-WWTP effluent (11 L) was collected in a 45 L (12 gallon) polypropylene container (submerged in an ice bath) by an Isco® sampler (Model 2700; Isco Inc., Lincoln, NE). The effluent was then siphoned into several containers provided for various analyses. The containers were placed on ice and delivered to Biospherics Inc. for analysis. Grab samples of diluent water were taken from a large polypropylene tank with a 99% particle replacement time of ≈12 h, placed in appropriate containers, and delivered on ice to Biospherics Inc. for analysis.

The materials analyzed in the effluent and diluent water and their quantitation limits are listed in Table 3. The analytical methods used by Biospherics Inc. for general water chemistry, metals, volatiles, semi-volatiles, PCB/pesticides, and herbicides for both the diluent water and effluent are given in Table 4.

3.8.2 Routine Water Quality Analyses - Carcinogenicity and Ventilatory Tests

Routine water quality analyses were conducted on grab samples taken from the carcinogenicity test aquaria and from the ventilatory dilutors in the biological monitoring early warning system as described in Sections 3.6 and 3.7, respectively. Dissolved oxygen, pH, and temperature were measured daily. Alkalinity, total ammonia-nitrogen, total residual and free available chlorine, and hardness were measured twice a week (all tests were performed together on the same days). The chemical analysis methods are summarized in Table 5. Un-ionized ammonia-nitrogen was determined by the method of Thurston et al. (1979). The following sampling schedule was used:

1) Sunday through Saturday - DO, pH, and temperature were measured in:

   a) All 12 aquaria in the carcinogenicity test.
   b) Effluent and diluent water in the 21-d ventilatory tests.

2) Tuesday - Alkalinity, ammonia-nitrogen, chlorine, and hardness were measured in:

   a) 6 even numbered aquaria in the carcinogenicity test.
   b) Effluent and diluent water in the 21-d ventilatory tests.

3) Friday - Alkalinity, ammonia-nitrogen, chlorine, and hardness were measured in:

   a) 6 odd numbered aquaria in the carcinogenicity test.
   b) Effluent and diluent water in the 21-d ventilatory tests.

In addition to the temperature measurements made via grab samples during the carcinogenicity test, temperature was monitored continuously in the water bath which held the exposure aquaria via a strip chart recorder (Cole-Parmer Thermistor Recorder Model No. 08354-15, Cole-Palmer Instrument Co., Chicago, IL). Temperature was also monitored continuously during each ventilatory test from 1) a thermistor placed in one of the ventilatory dilutor chambers and transduced to a strip chart recorder (same model as above) and 2) via the data acquisition system described below in Section 3.8.4.

3.8.3 Routine Water Quality Analyses - Microtox® Tests

Total residual and free available chlorine were measured in all 24-h composite samples used in the Microtox® assays beginning in October 1990. Total ammonia-nitrogen was measured twice a week beginning in December 1990; un-ionized ammonia-nitrogen was also calculated by the method of Thurston et al. (1979). The ammonia-nitrogen analysis was performed on 24-h composite effluent used for the Microtox® assays on Tuesday and Friday. Chlorine and ammonia-nitrogen concentrations were determined by the methods shown in Table 5.

3.8.4 Automated Water Quality Analyses

The following water quality parameters were continuously monitored at 30-minute intervals during the 21-d ventilation studies for both the effluent and diluent water: DO, pH, temperature, conductivity, and turbidity. The ventilatory data

acquisition system was programmed to record a 30 minute average measurement of each parameter in the effluent followed by a 30 minute average measurement of the parameters in the diluent water. A Hydrolab® Scout® (Hydrolab Corp., Austin, TX) was used to monitor DO, pH, temperature, and conductivity. A Hach® Surface Scatter 5 Turbidimeter (Hach Co., Loveland, CO) was used to monitor turbidity. As was the case for the ventilation data discussed in Section 3.7, the water quality data from each test were also transferred from the data acquisition system to floppy disks for subsequent analysis at USABRDL.

SECTION 4

RESULTS AND DISCUSSION

4.1 Acute Toxicity

4.1.1 Microtox®

The results of the Microtox® tests conducted from September 5, 1990 to February 12, 1991 are summarized in Table 6. Acute toxicity was found in ≈10% of the daily effluent samples analyzed (toxicity occurred 16 days out of 156 days of sampling). Nine 5-min EC50s and ten 15-min EC50s were measured. Three samples gave both 5- and 15-min readings. The 5-min EC50s ranged from 19.5 to 96.3 percent effluent by volume. The 15-min EC50s ranged from 20.9 to 96.3 percent effluent by volume. Toxicity appeared to occur in a random pattern over the test period; toxicity did occur two days consecutively in two cases.

Because 6 samples during the first 25 days that Microtox® measurements were made (September 5-30, 1990) were found to be toxic, chlorine measurements were initiated on October 4, 1990 to determine whether or not a correlation existed between Microtox® toxicity and the low concentrations of total residual chlorine (mean TRC = 0.06 mg/L; n = 106) present in the effluent (Table 6). No correlation was found between Microtox® toxicity and TRC concentrations. Beginning in mid-December, ammonia-nitrogen measurements were added to the testing suite. No correlation was found between Microtox® toxicity, chlorine concentrations, or ammonia-nitrogen concentrations (Table 6).

4.1.2 Rotifer Toxicity Test

The effluent (100% effluent) was not toxic to the rotifer in three separate tests. A synopsis of each test performed, mean water quality, rotifer survival, and statistical analysis of the data are given in Appendices 1-3.

4.2 Chronic Toxicity

4.2.1 Green Algal Growth Test

No toxicity occurred in the tests conducted with the green alga during the periods July 24-28, 1990 and November 8-12, 1990 (see Appendices 4 and 5). In contrast to the first two tests, a significant reduction (alpha = 0.05) in growth relative to the control organisms occurred in 100% effluent and in APG diluent water during the third test conducted February 12-16, 1991 (Appendix 6). No reduction in growth occurred in effluent treatments below 100% (Appendix 6; Table A6-3). A 96-h EC50 for reduction in growth could not be calculated using the probit analysis because a reduction occurred in only one concentration.

A synopsis of each test performed, cell density, growth rate, etc., are given in Appendices 4-6.

4.2.2 Daphnid Survival and Reproduction Test

APG-WWTP effluent had no affect on adult survival or neonate production at concentrations up to 100% effluent by volume in the first two tests conducted during the periods July 24-31, 1990 and November 5-12, 1990 (Appendices 7 and 8). The effluent did not affect the survival of the adults after 7 d of exposure in the third test conducted February 6-13, 1991; however, a statistically significant (alpha = 0.05) increase in neonate production occurred in 10% effluent by volume only (Appendix 9; Tables A9-2 and A9-3). The increase in neonate production is most likely attributable to statistical chance, i.e., 1 in 20 times one can expect a random event to occur. A synopsis of each test performed, mean water quality, adult survival, neonate production, and statistical analysis of the data are given in Appendices 7-9.

4.2.3 Fathead Minnow Survival and Growth Test

The effluent had no affect on larval survival at concentrations up to 100% during the first two tests conducted July 24-31, 1990 and November 5-12, 1991 (Appendices 10 and 11). Significant mortality relative to the JHU/APL-AES control organisms occurred to fathead minnow larvae exposed to APG diluent water and APG-WWTP effluent at concentrations above 6.25% effluent by volume during the third test conducted February 6-13, 1991 (Appendix 12; Tables A12-1 and A12-2).

The potential effect of the effluent on larval growth could not be determined during the first test conducted July 24-31, 1990 because the dry weight samples were lost due to a malfunction in a drying oven. During the second test, a statistically significant (alpha = 0.05) reduction in dry weight occurred at the 12.5% effluent by volume concentration only (Appendix 11; Tables A11-2 and A11-3). No difference was found at the 6.25% effluent by volume concentration or at any of the concentrations above 12.5% effluent by volume. It appears that the reduction in growth in the second study at 12.5% effluent by volume is most likely due to chance. Larval growth was not affected during the third study (Appendix 12; Table A12-3).

4.3 Mutagenicity

The results of the Ames mutagenicity assays conducted during the APG-WWTP study are summarized in Table 7. With the exception of the concentrated (10X) effluent samples taken on November 7, 1990, and February 6, 1991 (see below), none of the samples caused a positive increase in the numbers of histidine revertants per plate with tester strains TA98 or TA100 either in the

presence or absence of microsomal enzymes prepared from Aroclor 1254-induced rat liver.

The concentrated (10X) APG-WWTP effluent sample taken on November 7, 1990 caused a reproducible positive increase (2.7- and 4.1-fold) in the number of histidine revertants per plate with tester strain TA98 in the presence of S9. No positive increases were observed with tester strain TA98 in the absence of S9 or with tester strain TA100 in either the presence or absence of S9. The concentrated (10X) effluent sample obtained on February 6, 1991 caused a positive increase in the number of histidine revertants per plate with tester strain TA98 (2.2-fold in the initial mutagenicity assay and 2.1-fold in a confirmatory assay) in the presence of microsomal enzymes prepared from Aroclor 1254-induced rat liver. No positive increases in the number of histidine revertants per plate were observed with any of the remaining tester strain/activation combinations.

4.4 Teratogenicity

No data are available for the two FETAX assays conducted during the APG-WWTP study because the assays were preliminary rather that definitive assays.

4.5 Carcinogenicity

Detailed results of carcinogenicity test (T) are given in Botts (1992). Routine water quality during the exposure period is discussed in Section 4.7.2. Briefly, the pathological results as summarized by Botts (1992) are as follows. Liver neoplasms and foci of cellular alteration occurred at a slightly higher incidence in fish in the groups exposed to APG-WWTP effluent than in APG diluent water controls at the interim (day 121 of exposure), chronic (day 200 of exposure), and recovery observation periods. Incidence of the lesions were similar in control fish held for the same length of time in USABRDL well water at Ft. Detrick, Frederick, MD. Hepatic vacuolation and cystic degeneration occurred in several fish in most groups at all three observation periods; a slight increase in the severity of these lesions was observed in the fish exposed to effluent at the 121- and 200-d observation periods.

Changes in kidney and thyroid tissue occurred in fish at each observation period of the study and in all groups; however, no apparent pattern of incidence was present which could be related to exposure. Nonhepatic neoplasms which occurred infrequently, but only in fish exposed to APG-WWTP effluent, included lymphosarcoma, mesothelioma, ocular medulloepithelioma, ovarian teratoma, gas gland epithelioma, and thyroid follicular cell neoplasms.

4.6 Biological Monitoring Early Warning System

No abrupt changes in effluent quality or episodic events were detected during Ventilatory Tests I and II. The effluent was not overtly toxic to the bluegills during the 14-d definitive phases of each test. That is, significant mortality did not occur during 14 d of exposure to 100% effluent. Mr. Tommy R. Shedd of USABRDL may be contacted for further information concerning ventilation frequency, opercular amplitude, cough frequency, etc, obtained during the two studies.

4.7 Chemical Analyses

4.7.1 Comprehensive Chemical Analyses

The results of the four comprehensive chemical analyses of the APG-WWTP effluent and APG diluent water are summarized in Table 8. The only values reported are for those chemicals whose concentrations were at or above the quantitation limits given in Table 3. Lead, one of eight heavy metal priority pollutants (Section 307 toxic pollutants) measured in this study, was found in two (September 27, 1990 and November 7, 1990) of the four effluent samples. Of the eight heavy metal priority pollutants measured (Note: there are 12 heavy metal priority pollutants), no other heavy metal priority pollutant was detected in the effluent or diluent water.

Several organic priority pollutants were detected in the effluent (Table 8). The following volatiles were detected in the February 6, 1991 sample: benzene, ethylbenzene, and toluene. Chloroform was detected in the September 27, 1990 and November 7, 1990 effluent samples. Bis(2-ethylhexyl)phthalate and phenol, both semi-volatile compounds, were detected in the February sample. Bis(2-ethylhexyl)phthalate was also detected in the September effluent sample. The following pesticides were detected in the sample taken July 24, 1990: dieldrin, endosulfan sulfate, endrin, and methoxychlor. The herbicide, 2,4-D, was detected only once in the February 6, 1991 effluent sample. Aldrin was the only priority pollutant found in the diluent water (September 27, 1990).

4.7.2 Routine Water Quality Analyses

The mean water quality in each of the 12 carcinogenicity test aquaria for the period August 22, 1990 to February 13, 1991 is summarized in Table 9. The chlorine and ammonia-nitrogen data taken in support of the Microtox® tests (Section 4.1.1) are given in Table 6. Mr. Tommy R. Shedd of UASBRDL may be contacted for the routine water quality data taken via grab samples during the biological monitoring early warning system tests.

4.7.3 Automated Water Quality Analyses

Mr. Tommy R. Shedd of UASBRDL may be contacted for the automated routine water quality data logged during the two biological monitoring early warning system tests.

SECTION 5

CONCLUSIONS

The array of biological monitoring techniques used to assess the potential toxicity of the APG-WWTP effluent showed that the effluent generally was not toxic during most of the study period. Toxicity was detected by the following test systems. Acute toxicity was found in ≈10% of the effluent samples measured (toxicity occurred 16 days out of 156 days of sampling) via Microtox®. Toxicity appeared to occur in a random pattern over the 6-month test period; toxicity did occur two days consecutively in two cases. The cause of the acute toxicity measured by Microtox® was not obvious. For example, no correlation was found between Microtox® toxicity, chlorine concentrations, or ammonia-nitrogen concentrations. No acute toxicity was found in the 24-h rotifer tests.

Chronic toxicity was detected during the February 1991 series of tests in two of the three biomonitoring systems used. A significant reduction in growth occurred in the algal growth test in 100% effluent; no toxicity occurred at lower test concentrations. Significant mortality occurred to fathead minnow larvae exposed to APG diluent water and APG-WWTP effluent at concentrations above 6.25% effluent by volume during the third test. The comprehensive chemical analysis conducted on one 24-h composite sample taken during the February 1991 period when chronic toxicity occurred showed that several volatiles, semi-volatiles and 2,4-D were present in the effluent which were not present in prior effluent samples. However, the concentration of each priority pollutant was substantially below the water quality criterion concentration for each compound (USEPA, 1986). It is not clear whether or not the combined effect of all the compounds may have contributed to the toxicity observed in the two tests. No chronic toxicity was found using the algal, daphnid, or fathead minnow tests during the July 1990 and November 1990 test periods.

No mutagenicity was detected in unconcentrated APG-WWTP effluent, unconcentrated dechlorinated APG diluent water or concentrated (10X) dechlorinated APG diluent water. Mutagenicity was found in the November 1990 and February 1991 effluent samples which were concentrated 10X; no mutagenicity was observed in the September 1990 concentrated (10X) effluent sample. No definitive teratogenicity data are available because only preliminary tests where conducted.

The following carcinogenicity events were found in the study. Liver neoplasms and foci of cellular alteration occurred at a slightly higher incidence in fish in the groups exposed to APG-WWTP effluent than in APG diluent water controls at the

interim (day 121 of exposure), chronic (day 200 of exposure), and recovery observation periods. Incidence of these lesions were similar in control fish held for the same length of time in USABRDL well water at Ft. Detrick, Frederick, MD. Hepatic vacuolation and cystic degeneration occurred in several fish in most groups at all three observation periods; a slight increase in the severity of the lesions was observed in the fish exposed to effluent at the 121- and 200-d observation periods.

Changes in kidney and thyroid tissue occurred in fish at each observation period of the study and in all groups; however, no apparent pattern of incidence was present which could be related to exposure. Nonhepatic neoplasms which occurred infrequently, but only in fish exposed to APG-WWTP effluent, included lymphosarcoma, mesothelioma, ocular medulloepithelioma, ovarian teratoma, gas gland epithelioma, and thyroid follicular cell neoplasms.

The two biological monitoring early warning system tests showed that no abrupt changes in effluent quality or episodic events occurred. The effluent was not overtly toxic to the bluegills during the 14-d definitive phases of each test. That is, significant mortality did not occur during 14 d of exposure to 100% effluent.

SECTION 6

REFERENCES

APHA et al. (American Public Heath Association, American Water Works Association, and Water Pollution Control Federation). 1989. Standard methods for the examination of water and wastewater, 17th ed. Amer. Public Hlth. Assoc., Washington, DC.

Bantle, J.S. and T.D. Sabourin. 1990. Proposed new standard guide for conducting the frog embryo teratogenesis assay - Xenopus (FETAX). Draft No. 3. Amer. Soc. Testing Materials, Philadelphia, PA.

Botts, S. 1992. Aberdeen Proving Ground (APG) wastewater treatment plant effluent study, test T. Draft Report. Experimental Pathology Laboratories, Inc., Herdon, VA.

Hawkins, W.E., R.M. Overstreet, and W.W. Walker. 1988. Small fish models for identifying carcinogens in the aqueous environment. Water Resour. Bull. 24:941-949.

Herriott, R.S. and D.T. Burton. 1992. U.S. Army Biomedical Research and Development Laboratory Aquatic Biomonitoring Trailer Version 1.0: Operations Manual. Draft Final Report. Rep. No. WREC-92-B3, University of Maryland Wye Research and Education Center, Queenstown, MD.

Horning, W.B., II and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory-Cincinnati, Cincinatti, OH.

Klaunig, J.E., B.A. Barut, and P.J. Goldblatt. 1984. Preliminary studies on the usefulness of medaka, Oryzias latipes, embryos in carcinogenicity testing. Natl. Cancer Inst. Monogr. 65:155-161.

Logan, E. 1992. Personal communication. Aberdeen Proving Ground, Aberdeen, MD.

Metcalfe, C.D. 1989. Tests for predicting carcinogenicity in fish. Rev. Aquatic Sci. 1:111-129.

Microtox®. 1988. Microtox® Manual Model 500 Toxicity Test System. Microbics Corp., Carlsbad, CA.

Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz algal assay bottle test. Experimental design, application and data interpretation protocol. EPA-600/9-78-018. U.S. Environmental Protection Agency, Environmental Research Laboratory-Corvallis, Corvallis, OR.

Peltier, W.H. and C.I. Weber. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd ed. EPA/600/4-85/013. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory-Cincinnati, Cincinatti, OH.

Shedd, T.R., W.H. van der Schalie, and M.G. Zeeman. 1986. Evaluation of an automated fish ventilatory monitoring system in a short-term screening test for chronic toxicity. AD-A172116. U.S. Army Medical Bioengineering Research and Development Laboratory, Ft. Detrick, Frederick, MD.

Thurston, R.V., R.C. Russo, and K. Emerson. 1979. Aqueous ammonia equilibrium - Tabulation of percent ionized ammonia. EPA-600/3-79-091. U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, MN.

USABRDL. 1991. Aquatic biomonitoring program user's guide manual - Version 2.0. U.S. Army Biomedical Research and Development Laboratory (USABRDL), Ft. Detrick, Frederick, MD.

USEPA. 1983. Methods for chemical analysis of water and wastes. EPA 600/4-79/020 (Revised 1983). U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory-Cincinnati, OH.

USEPA. 1986. Quality criteria for water 1986. EPA 440/5-86-001. U.S. Environmental Protection Agency, Office of Regulations and Standards, Washington, DC.

Waller, T. and J. Lazorchak. 1986. Proposed new standard guide for conducting three brood, renewal toxicity tests with Ceriodaphnia dubia. Draft No. 3. Amer. Soc. Testing Materials, Philadelphia, PA.

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning, II, F.A. Kessler, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer, and R.W. Freyberg. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd ed. EPA/600/4-89/001. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory-Cincinnati, Cincinatti, OH.

Winner, R.W. 1987. Personal communication. Miami Univ., Oxford, OH.

Winner, R.W. 1989. Multigeneration life-span tests of the nutritional adequacy of several diets and culture waters for *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 8:513-520.

TABLE 1. SUMMARY OF THE BIOMONITORING TESTS CONDUCTED.

Test and/or Species	Type of Test	Test Periods	Comments
Microtox® (Bacterium)	5- and/or 15-min EC50	09/05/90 - 02/12/91	Daily 24-h composite samples
ToxKit™ (Rotifer)	24-h LC50	07/25/90 - 07/27/90 11/10/90 - 11/12/90 02/08/91 - 02/10/91	24-h composite sample
Green alga	96-h EC50	07/24/90 - 07/28/90 11/08/90 - 11/12/90 02/12/91 - 02/16/91	24-h composite sample
Daphnid	7-d Survival and reproduction	07/24/90 - 07/31/90 11/06/90 - 11/13/90 02/06/91 - 02/13/91	24-h composite samples renewed every 24 h
Fathead minnow	7-d Survival and growth	07/24/90 - 07/31/90 11/06/90 - 11/12/91 02/06/91 - 02/13/91	24-h composite samples renewed every 24 h
Mutagenicity (Bacterium)	Ames assay	09/27/90 11/07/90 02/06/91	24-h composite sample
Teratogenicity (African frog)	4-d FETAX	09/10/90 - 09/14/90[a] 12/03/90 - 12/07/90[a]	Flow-through exposure
Carcinogenicity (Japanese medaka)	6-months	08/22/90 - 02/13/91	Flow-through exposure
Bluegill early warning system	21-d	07/21/90 - 08/10/90 10/29/90 - 11/19/90	Flow-through exposure

TABLE 1. (CONTINUED).

Test and/or Species	Type of Test	Test Periods	Comments
Comprehensive chemical analyses	N/A	07/24/90 09/27/90 11/07/90 02/06/91	24-h composite sample
Routine water quality analyses	N/A		See text

* Preliminary test only.

TABLE 2. COMPREHENSIVE WATER CHEMISTRY ANALYSIS OF THE JHU/APL-AES WELL WATER.

Base/Neutrals

Compound	ug/L[a]	Compound	ug/L[a]
Bis(2-chloroethyl)ether		Di-n-butylphthalate	
1,3-Dichlorobenzene		Flouranthene	
1,4-Dichlorobenzene		Pyrene	
1,2-Dichlorobenzene		Butylbenzylphthalate	
Bis(2-chloroisopropyl)ether		3,3'-Dichlorobenzidine	
N-Nitroso-di-n-propylamine		Benzo(a)anthracene	
Hexachloroethane		Bis(2-ethylhexyl)phthalate	
Nitrobenzene		Chrysene	
Isophorone		Di-n-octylphthalate	
Bis(2-chloroethoxy)methane		Benzo(b)fluoranthene	
1,2,4-Trichlorobenzene		Benzo(k)fluoranthene	
Naphthalene		Benzo(a)pyrene	
Hexachlorobutadiene		Indeno(1,2,3-cd)pyrene	
Hexachlorocyclopentadiene		Dibenzo(a,h)anthracene	
2-Chloronaphthalene		Benzo(g,hi,i)perylene	
Dimethylphthalate			
Acenaphthylene		The following are non-priority pollutant hazardous substance list compounds:	
Acenaphthene			
2,4-Dinitrotoluene			
2,6-Dinitrotoluene		Aniline	
Diethylphthalate		Benzyl Alcohol	
4-Chlorophenyl-phenylether		4-Chloroaniline	
Fluorene		2-Methylnaphthalene	
N-Nitrosodiphenylamine		2-Nitroaniline	
4-Bromophenyl-phenylether		3-Nitroaniline	
Hexachlorobenzene		Dibenzofuran	
Phenanthrene		4-Nitroaniline	
Anthracene			
		DETECTION LIMIT	2

TABLE 2. (CONTINUED).

Pesticides		Metals	
Compound	**ug/L[a]**	**Metal (Total)**	**mg/L**
Alpha-BHC		Antimony	<0.005
Beta-BHC		Arsenic	<0.005
Delta-BHC		Beryllium	<0.005
Gamma-BHC (Lindane)		Cadmium	<0.001
Heptachlor		Chromium	<0.05
Aldrin		Copper	<0.02
Heptachlor epoxide		Mercury	<0.0002
Alpha-endosulfan		Lead	<0.005
Dieldrin		Nickel	<0.20
4,4'-DDE		Selenium	<0.005
Endrin		Silver	<0.01
Beta-endosulfan		Thallium	<0.005
4,4'-DDD		Zinc	<0.04
Endrin aldehyde			
Endosulfan sulfate		**Water Quality**	**mg/L**
4,4'-DDT			
Methoxychlor		Alkalinity (as $CaCO_3$)	156
Chlordane		Ammonia (as N)	0.15
Toxaphene		Hardness (as $CaCO_3$)	190
Aroclor 1016		Nitrate	<0.10
Aroclor 1221		Nitrite	<0.10
Aroclor 1232		Total Kjeldahl Nitrogen	0.15
Aroclor 1242		Total Organic Carbon	19
Aroclor 1248			
Aroclor 1254		pH	7.8
Aroclor 1260			
DETECTION LIMIT	0.1		

[a] Concentrations less than the detection limit are left blank.

TABLE 3. APG-WWTP EFFLUENT AND APG DILUENT WATER CHEMICAL CHARACTERISTICS AND THEIR QUANTITATION LIMITS - GENERAL WATER QUALITY.

Parameter	Quantitation Limits (mg/L)
Alkalinity (as $CaCO_3$)	5.0[a]
Ammonia (as N)	0.02[b]
Cyanide	0.01
Hardness (as $CaCO_3$)	N/A
Nitrite	0.05[c]
Nitrate	0.05[c]
Nitrate/nitrite combined as N	0.1[d]
Phosphorous	0.02
Sulfide	2.0
Conductivity (umho/cm)	N/A
Total suspended solids	5.0
Fluoride	0.10
Sulfate	1.0
Total organic carbon	1.0[e]
Residual chlorine	0.1

TABLE 3. (CONTINUED) - METALS.

Parameter	Quantitation Limits (mg/L)
Aluminum	0.2
Arsenic	0.01[f]
Barium	0.05
Beryllium	0.005
Boron	0.1[g]
Cadmium	0.005
Calcium	5.0[h]
Cobalt	0.05
Copper	0.025
Iron	0.10
Lead	0.005
Magnesium	1.0
Manganese	0.015
Mercury	0.0005
Molybdenum	0.05
Nickel	0.04
Potassium	1.0[i]
Selenium	0.005
Silver	0.01
Sodium	1.0

TABLE 3. (CONTINUED) - VOLATILE ORGANICS.

C.A.S. Number	Compound Name	Quantitation Limits (ug/L)
74-87-3	Chloromethane	10.0
74-83-9	Bromomethane	10.0
75-01-4	Vinyl chloride	10.0
75-00-3	Chloroethane	10.0
75-09-2	Methylene chloride	5.0
67-64-1	Acetone	100.0
75-69-4	Trichlorofluoromethane	5.0
75-15-0	Carbon disulfide	5.0
107-02-8	Acrolein	50.0
107-13-1	Acrylonitrile	50.0
75-35-4	1,1-Dichloroethene	5.0
75-34-3	1,1-Dichloroethane	5.0
540-59-0	Trans-1,2-dichloroethene	5.0
67-66-3	Chloroform	5.0
107-06-2	1,2-Dichloroethane	5.0
78-93-3	2-Butanone	100.0
71-55-6	1,1,1-Trichloroethane	5.0
56-23-5	Carbon tetrachloride	5.0
108-05-4	Vinyl acetate	50.0[j]
75-27-4	Bromodichloromethane	5.0
78-87-5	1,2-Dichloropropane	5.0
10061-01-5	Cis-1,3-dichloropropene	5.0
79-01-6	Trichloroethene	5.0
124-48-1	Dibromochloromethane	5.0
79-00-5	1,1,2-Trichloroethane	5.0
71-43-2	Benzene	5.0
10061-02-6	Trans-1,3-dichloropropene	5.0
110-75-8	2-Chloroethylvinylether	10.0
108-10-1	4-Methyl-2-pentanone	50.0
591-78-6	2-Hexanone	50.0
75-25-2	Bromoform	5.0
127-18-4	Tetrachloroethene	5.0
79-34-5	1,1,2,2-Tetrachloroethane	5.0
108-88-3	Toluene	5.0
108-90-7	Chlorobenzene	5.0
100-41-4	Ethylbenzene	5.0
100-42-5	Styrene	5.0
1330-20-7	Total xylenes	5.0

TABLE 3. (CONTINUED) - SEMI-VOLATILE ORGANICS.

C.A.S. Number	Compound Name	Quantitation Limits (ug/L)
62-75-9	N-Nitrosodimethylamine	10.0
108-95-2	Phenol	10.0
111-44-4	Bis(-2-chloroethyl)ether	10.0
95-57-8	2-Chlorophenol	10.0
541-73-1	1,3-Dichlorobenzene	10.0
106-46-7	1,4-Dichlorobenzene	10.0
100-51-6	Benzyl alcohol	10.0
95-50-1	1,2-Dichlorobenzene	10.0
95-48-7	2-Methylphenol	10.0
39638-32-9	Bis(2-chloroisopropyl)ether	10.0
106-44-5	4-Methylphenol	10.0
621-64-7	N-Nitroso-di-n-propylamine	10.0
67-72-1	Hexachloroethane	10.0
98-95-3	Nitrobenzene	10.0
78-59-1	Isophorone	10.0
88-75-5	2-Nitrophenol	10.0
105-67-9	2,4-Dimethylphenol	10.0
65-85-0	Benzoic acid	50.0
111-91-1	Bis(-2-chloroethoxy)methane	10.0
120-83-2	2,4-Dichlorophenol	10.0
120-82-1	1,2,4-Trichlorobenzene	10.0
91-20-3	Naphthalene	10.0
106-47-8	4-Chloroaniline	10.0
87-68-3	Hexachlorobutadiene	10.0
59-50-7	4-Chloro-3-methylphenol	10.0
91-57-6	2-Methylnaphthalene	10.0
77-47-4	Hexachlorocyclopentadiene	10.0
88-06-2	2,4,6-Trichlorophenol	10.0
95-95-4	2,4,5-Trichlorophenol	50.0
91-58-7	2-Chloronaphthalene	10.0
88-74-4	2-Nitroaniline	50.0
131-11-3	Dimethyl phthalate	10.0
208-96-8	Acenaphthylene	10.0
99-09-2	3-Nitroaniline	50.0
83-32-9	Acenaphthene	10.0
51-28-5	2,4-Dinitrophenol	50.0
100-02-7	4-Nitrophenol	50.0
132-64-9	Dibenzofuran	10.0
606-20-2	2,6-Dinitrotoluene	10.0
121-14-2	2,4-Dinitrotoluene	10.0
84-66-2	Diethylphthalate	10.0
7005-72-3	4-Chlorophenyl-phenylether	10.0
86-73-7	Fluorene	10.0

TABLE 3. (CONTINUED) - SEMI-VOLATILE ORGANICS CON'T.

C.A.S. Number	Compound Name	Quantitation Limits (ug/L)
100-01-6	4-Nitroaniline	50.0
86-30-6	N-Nitrosodiphenylamine	10.0
103-33-3	1,2-Diphenylhydrazine	10.0
101-55-3	4-Bromophenyl-phenylether	10.0
87-86-5	Pentachlorophenol	50.0
85-01-8	Phenanthrene	10.0
120-12-7	Anthracene	10.0
84-74-2	Di-n-butylphthalate	10.0
118-74-1	Hexachlorobenzene	10.0
206-44-0	Fluoranthene	10.0
92-87-5	Benzidine	50.0
129-00-0	Pyrene	10.0
85-68-7	Butylbenzylphthalate	10.0
91-94-1	3,3'-Dichlorobenzidine	20.0
56-55-3	Benzo(a)anthracene	10.0
117-81-7	Bis(2-ethylhexyl)phthalate	10.0
218-01-9	Chrysene	10.0
117-84-0	Di-n-octyl phthalate	10.0
205-99-2	Benzo(b)fluoranthene	10.0
207-08-9	Benzo(k)fluoranthene	10.0
50-32-8	Benzo(a)pyrene	10.0
193-39-5	Indeno(1,2,3-cd)pyrene	10.0
53-70-3	Dibenzo(a,h)anthracene	10.0
191-24-2	Benzo(g,h,i)perylene	10.0

TABLE 3. (CONTINUED) - PESTICIDES/PCBs AND HERBICIDES.

C.A.S. Number	Parameter	Quantitation Limits (ug/L)
	Pesticide/PCB	
319-84-6	Alpha-BHC	0.02
319-87-7	Beta-BHC	0.02
319-86-8	Delta-BHC	0.02
58-89-9	Lindane	0.02
76-44-8	Heptachlor	0.02
309-00-2	Aldrin	0.02
1024-57-3	Heptachlor epoxide	0.02
959-98-8	Endosulfan I	0.02
60-57-1	Dieldrin	0.02
75-55-9	4,4'-DDE	0.02
72-20-8	Endrin	0.02
33213-65-9	Endosulfan II	0.02
72-54-8	4,4'-DDD	0.02
1031-07-8	Endosulfan sulfate	0.02
50-29-3	4,4'-DDT	0.02
72-43-5	Methoxychlor	0.02
7421-93-4	Endrin aldehyde	0.02
57-74-9	Chlordane	0.16[k]
8001-35-2	Toxaphene	1.0[l]
12674-11-2	Aroclor-1016	0.20
11104-28-2	Aroclor-1221	0.20
11141-16-5	Aroclor-1232	0.20
53469-21-9	Aroclor-1242	0.20[m]
12672-29-6	Aroclor-1248	0.20[m]
11097-69-1	Aroclor-1254	0.20
11096-82-5	Aroclor-1260	0.20
53469-21-9	Aroclor-1248	0.20[n]
	Herbicide	
94-75-7	2,4-D	0.1[o]
93-72-1	Silvex	0.1[o]
93-76-5	2,4,5-T	0.1[o]

[a] Alkalinity was not measured during the 2/6/91 analysis.
[b] Quantitation limit was 0.1 mg/L for both the diluent and effluent samples during the 7/24/90 analysis and 0.3 mg/L for the effluent sample during the 9/27/90 analysis only.
[c] Measured separately during the 7/24/90 analysis only.
[d] Nitrate/nitrite combined as N was not measured during the 7/24/90 and 2/6/91 analyses.
[e] Quantitation limit was 3.0 mg/L for the effluent sample during the 9/27/90 analysis only.

TABLE 3. (CONTINUED) - FOOTNOTES CON'T.

[f] Quantitation limit was 0.005 mg/L during the 2/6/91 analysis only.
[g] Quantitation limit was 0.050 mg/L during the 11/7/90 and 2/6/91 analyses.
[h] Quantitation limit was 1.0 mg/L during the 2/6/91 analysis only.
[i] Quantitation limit was 0.1 mg/L during the 2/6/91 analysis only.
[j] Quantitation limit was 10.0 ug/L during the 2/6/91 analysis only.
[k] Practical quantitation limit was 0.02 ug/L during the 7/24/90 analysis only.
[l] Practical quantitation limit was 0.02 ug/L during the 7/24/90 analysis only.
[m] Not analyzed during the 9/27/90 analysis.
[n] Analyzed during the 9/27/90 analysis only.
[o] Practical quantitation limit for the effluent sample was 1.0 ug/L during the 2/6/91 analysis only.

TABLE 4. SUMMARY OF THE ANALYTICAL METHODS USED FOR THE APG-WWTP EFFLUENT, APG DILUENT WATER, AND JHU/APL-AES DILUENT WATER CHEMICAL ANALYSES.

Parameter	Method	Reference
Metals	EPA 200.0's/200.7	(USEPA, 1983)
Mercury	EPA 245.1	(USEPA, 1983)
Alkalinity	EPA 310.1	(USEPA, 1983)
Ammonia	EPA 350.2/350.1[a]	(USEPA, 1983)
Cyanide	EPA 335.2	(USEPA, 1983)
Phosphorous	EPA 365.2	(USEPA, 1983)
Sulfide	EPA 376.1	(USEPA, 1983)
Nitrate+Nitrite	EPA 353.2	(USEPA, 1983)
Nitrite	EPA 353.2	(USEPA, 1983)
Conductivity	EPA 120.1	(USEPA, 1983)
Total suspended solids	EPA 160.1	(USEPA, 1983)
Fluoride	EPA 340.2	(USEPA, 1983)
Sulfate	EPA 375.4/300.1/300.0[b]	(USEPA, 1983)
Residual chlorine	Hach kit	Hach Co.[c]
Hardness	Standard Methods 2340 B	(APHA et al., 1989)
Volatile organics	EPA 8240	(USEPA, 1983)
Semi-volatile organics	EPA 625	(USEPA, 1983)
Herbicide	EPA 615	(USEPA, 1983)
Total organic carbon	EPA 415.1/415.2[d]	(USEPA, 1983)
Pesticide/PCB	EPA 608	(USEPA, 1983)

[a] EPA 350.1 method used during 2/6/91 analysis only.
[b] EPA 300.1 and 300.0 methods used during the 7/24/90 and 2/6/91 analyses, respectively.
[c] Hach Co. 1985. Free and total chlorine test kit. Hach Company, Loveland, CO.
[d] EPA 415.2 method used during the 2/6/92 analysis only.

TABLE 5. ROUTINE WATER QUALITY ANALYSES AND METHODS OF ANALYSIS FOR ALL GRAB SAMPLES TAKEN IN THE BIOMONITORING TRAILER AND ALL SAMPLES ANALYZED AT THE JHU/APL-AES LABORATORY.

Parameter	Method[a]
Alkalinity	Method 2320 B. Titration Method
Ammonia-nitrogen	Method 4500-NH_3. Ammonia Selective Electrode Method
Chlorine	Method 4500-Cl G. DPD Colorimetric Method
Conductivity	Method 2510 B. Laboratory Method
Dissolved Oxygen	Method 4500-O G. Membrane Electrode Method
Hardness	Method 2340 C. EDTA Titrimetric Method
pH	Method 4500-H^+ B. Electrometric Method
Temperature	Method 2550 B. Laboratory and Field Methods

[a] All methods taken from Standard Methods (APHA et al., 1989).

TABLE 6. MICROTOX®, CHLORINE, AND AMMONIA-NITROGEN TEST RESULTS ON 24-HOUR COMPOSITE SAMPLES OF APG-WWTP EFFLUENT.

Date of Sample	Microtox®		Chlorine		Ammonia-Nitrogen	
	5-min EC50[a]	15-min EC50[a]	Total (mg/L)[b]	Free (mg/L)[b]	Total (mg/L)	Un-ionized (mg/L)
Sep 05	-	-				
06	88.9	-				
07	-	-				
08	-	-				
09	-	-				
10	82.3	82.7				
11	-	-				
12	-	-				
13	-	-				
14	-	-				
15	-	-				
16	-	-				
17	-	72.8				
18	-	-				
19	-	-				
20	-	-				
21	-	84.8				
22	19.5	20.9				
23	-	-				
24	-	80.5				
25	-	-				
26	-	-				
27	-	-				
28	-	-				
29	-	-				
30	-	-				

TABLE 6. (CONTINUED).

Date of Sample		Microtox®		Chlorine		Ammonia-Nitrogen	
		5-min EC50[a]	15-min EC50[a]	Total (mg/L)[b]	Free (mg/L)[b]	Total (mg/L)	Un-ionized (mg/L)
Oct	01	c	c				
	02	c	c				
	03	c	c				
	04	-	97.5	0.10	<0.02		
	05	-	-	0.08	<0.02		
	06	-	-	0.07	-		
	07	-	-	0.08	<0.02		
	08	-	-	0.09	<0.02		
	09	-	-	0.09	<0.02		
	10	-	-	0.08	<0.02		
	11	-	-	0.10	<0.02		
	12	-	-	0.08	<0.02		
	13	-	-	0.06	<0.02		
	14	-	-	0.07	<0.02		
	15	61.1	-	0.08	<0.02		
	16	-	-	<0.05	-		
	17	-	-	0.07	<0.02		
	18	-	-	0.10	<0.02		
	19	-	-	-	-		
	20	-	-	0.07	<0.02		
	21	-	-	0.08	<0.02		
	22	-	-	0.07	<0.02		
	23	-	-	0.05	<0.02		
	24	-	-	0.06	-		
	25	-	-	0.06	<0.02		
	26	-	84.7	0.07	<0.02		
	27	-	-	0.06	<0.02		
	28	-	-	0.07	<0.02		

TABLE 6. (CONTINUED).

Date of Sample		Microtox® 5-min EC50[a]	Microtox® 15-min EC50[a]	Chlorine Total (mg/L)[b]	Chlorine Free (mg/L)[b]	Ammonia-Nitrogen Total (mg/L)	Ammonia-Nitrogen Un-ionized (mg/L)
Oct	29	-	-	0.06	<0.02		
	30	-	-	0.07	<0.02		
	31	-	-	0.07	<0.02		
Nov	01	-	-	0.06	<0.02		
	02	-	-	0.08	<0.02		
	03	-	-	0.07	-		
	04	-	-	0.08	<0.02		
	05	-	-	0.11	<0.02		
	06	-	-	0.10	<0.02		
	07	-	-	0.08	-		
	08	-	-	0.06	-		
	09	-	-	0.06	-		
	10	-	-	0.05	-		
	11	-	-	0.05	-		
	12	-	-	0.05	-		
	13	-	-	0.06	-		
	14	-	-	-	-		
	15	-	-	0.06	-		
	16	96.3	-	0.06	-		
	17	-	-	-	-		
	18	-	-	-	-		
	19	-	92.9	-	-		
	20	-	-	0.07	-		
	21	-	-	0.07	<0.02		
	22	-	-	0.07	<0.02		
	23	55.6	-	0.10	-		
	24	-	-	0.07	0.02		
	25	-	-	0.07	0.02		

TABLE 6. (CONTINUED).

Date of Sample		Microtox® 5-min EC50[a]	Microtox® 15-min EC50[a]	Chlorine Total (mg/L)[b]	Chlorine Free (mg/L)[b]	Ammonia-Nitrogen Total (mg/L)	Ammonia-Nitrogen Un-ionized (mg/L)
Nov	26	-	-	<0.05	-		
	27	-	-	0.03	-		
	28	-	-	0.02	-		
	29	-	-	0.04	-		
	30	-	-	0.03	-		
Dec	01	-	-	0.04	-		
	02	-	-	<0.02	-		
	03	-	-	0.03	-		
	04	-	-	-	-		
	05	-	-	-	-		
	06	-	-	0.02	-		
	07	-	-	0.03	-		
	08	-	-	0.02	-		
	09	-	-	0.02	-		
	10	-	-	-	-		
	11	-	-	-	-	12.35	0.05014
	12	-	-	0.02	-		
	13	-	-	0.03	-		
	14	81.9	-	0.03	<0.02	11.05	0.07768
	15	-	d	0.03	-		
	16	-	d	0.03	-		
	17	-	d	0.03	-		
	18	-	-	0.05	-	9.10	0.06152
	19	-	-	0.06	-		
	20	-	-	0.08	<0.02		
	21	-	-	0.08	<0.02	10.80	0.00647
	22	-	-	<0.02	-		
	23	-	-	0.02	-		

TABLE 6. (CONTINUED).

Date of Sample		Microtox® 5-min EC50[a]	Microtox® 15-min EC50[a]	Chlorine Total (mg/L)[b]	Chlorine Free (mg/L)[b]	Ammonia-Nitrogen Total (mg/L)	Ammonia-Nitrogen Un-ionized (mg/L)
Dec	24	-	-	0.02	-		
	25	-	-	0.04	-	•	•
	26	-	-	-	-		
	27	-	-	-	-		
	28	-	-	0.06	0.02	•	•
	29	-	-	0.05	<0.02		
	30	-	-	0.06	<0.02		
	31	-	-	-	-		
Jan	01	-	-	0.05	<0.02		
	02	-	-	0.04	<0.02		
	03	-	-	0.04	<0.02		
	04	-	-	0.06	0.02	10.35	0.07845
	05	-	-	0.04	<0.02		
	06	-	27.1	0.05	0.02		
	07	-	-	0.05	<0.02		
	08	-	-	0.05	<0.02	8.65	0.03849
	09	-	-	0.04	<0.02		
	10	-	-	0.04	<0.02		
	11	-	-	0.05	<0.02	9.95	0.06736
	12	-	-	0.03	<0.02		
	13	95.9	-	0.05	<0.02		
	14	90.2	96.3	0.06	<0.02		
	15	-	-	0.05	0.02	8.95	0.03231
	16	-	-	0.05	<0.02		
	17	-	-	0.05	0.02		
	18	-	-	0.05	<0.02	6.20	0.02654
	19	-	-	0.04	<0.02		
	20	-	-	0.05	0.02		

TABLE 6. (CONTINUED).

Date of Sample		Microtox®: 5-min EC50[a]	Microtox®: 15-min EC50[a]	Chlorine: Total (mg/L)[b]	Chlorine: Free (mg/L)[b]	Ammonia-Nitrogen: Total (mg/L)	Ammonia-Nitrogen: Un-ionized (mg/L)
Jan	21	-	-	0.05	0.02		
	22	-	-	0.05	0.02	9.10	0.04113
	23	-	-	0.05	<0.02		
	24	-	-	0.05	0.02		
	25	-	-	0.05	0.02	14.50	0.07199
	26	-	-	f	f		
	27	-	-	f	f		
	28	-	-	0.05	0.02		
	29	-	-	f	f	13.25	0.07963
	30	-	-	f	f		
	31	-	-	f	f		
Feb	01	-	-	f	f	14.50	0.10215
	02	-	-	f	f		
	03	-	-	f	f		
	04	-	-	f	f		
	05	-	-	f	f	13.45	0.07774
	06	-	-	0.05	0.02		
	07	-	-	0.05	0.02		
	08	-	-	0.03	<0.02	11.15	0.06389
	09	-	-	0.05	0.02		
	10	-	-	0.05	0.02		
	11	-	-	0.05	<0.02		
	12	-	-	0.04	<0.02	12.65	0.07653
	13			TEST COMPLETED			

TABLE 6. (CONTINUED).

Statistical Parameters	Microtox® 5-min EC50[a]	Microtox® 15-min EC50[a]	Chlorine Total (mg/L)[b]	Chlorine Free (mg/L)[b]	Ammonia-Nitrogen Total (mg/L)	Ammonia-Nitrogen Un-ionized (mg/L)
Mean	[g]	[g]	0.06	0.02	11.000	0.05950
Standard Deviation	[g]	[g]	0.020	0.000	2.2595	0.024086
Minimum Value	[g]	[g]	<0.02	<0.02	6.200	0.00647
Maximum Value	[g]	[g]	0.11	0.02	14.500	0.10215
N	[g]	[g]	106	17	16	16

[a] EC50s are expressed as percent effluent by volume.
[b] Values preceded by a < sign were not used in the mean, standard deviation, and N statistics.
[c] Data not obtained because of software failure.
[d] Instrument indicated that too much light entered the read turret; therefore, results could not be obtained.
[e] Ammonia-nitrogen could not measured because the electrode would not stabilize.
[f] Chlorine readings could not be taken due to the presence of Rhodamine dye added to the effluent during a tracing study.
[g] Statistical analyses cannot be performed on the Microtox® EC50 values.

TABLE 7. SUMMARY OF THE AMES MUTAGENICITY ASSAY RESULTS.

Date of Sample	Parameter	Result
09/27/90	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	No mutagenic activity
	Diluent water- unconcentrated	No mutagenic activity
	Diluent water- concentrated (10X)	No mutagenic activity
11/07/90	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
	Diluent water- unconcentrated	No mutagenic activity
	Diluent water- concentrated (10X)	No mutagenic activity
02/06/91	Effluent- unconcentrated	No mutagenic activity
	Effluent- concentrated (10X)	Mutagenic activity[a]
	Diluent water- unconcentrated	No mutagenic activity
	Diluent water- concentrated (10X)	No mutagenic activity

[a] Refer to Section 4.3 for further information.

TABLE 8. RESULTS OF THE APG-WWTP EFFLUENT AND APG DILUENT WATER COMPREHENSIVE CHEMICAL ANALYSES - GENERAL WATER QUALITY.[a]

Parameter	Date of Sample			
	07/24/90	09/27/90	11/07/90	02/06/91
		Effluent		
Alkalinity	102	61	55	[b]
Ammonia-nitrogen	17.0	10.0	9.97	33.2
Cyanide	0.047	<0.02	0.036	<0.01
Hardness	140	141	132	142
Nitrite	<0.05	[b]	[b]	[b]
Nitrate	2.62	[b]	[b]	[b]
Nitrate/nitrite combined as N	[b]	6.6	10.0	[b]
Phosphorous	0.36	0.37	<0.02	0.13
Sulfide	<2	174	89	38
Conductivity (umho/cm)	694	719	654	[b]
Total suspended solids	6	<5	6	[b]
Fluoride	0.62	1.1	0.7	0.61
Sulfate	53	42	32	64
Total organic carbon	13	33	14	11
Residual chlorine	<0.01	<0.01	<0.06	<0.01

TABLE 8. (CONTINUED) - GENERAL WATER QUALITY.[a]

Parameter	Date of Sample			
	07/24/90	09/27/90	11/07/90	02/06/91
		Diluent Water		
Alkalinity	23	15	30	[b]
Ammonia-nitrogen	<0.01	0.02	1.16	0.06
Cyanide	<0.01	<0.02	<0.01	<0.01
Hardness	79	71	74	61
Nitrite	0.72	[b]	[b]	[b]
Nitrate	0.51	[b]	[b]	[b]
Nitrate/nitrite combined as N	[b]	3.0	3.6	[b]
Phosphorous	0.27	0.06	0.28	0.13
Sulfide	<2	181	141	8
Conductivity (umho/cm)	311	238	225	[b]
Total suspended solids	<5	<5	<5	[b]
Fluoride	0.75	1.6	0.8	0.65
Sulfate	36	21	19	19
Total organic carbon	<1	6	<1	<1
Residual chlorine	<0.1	<0.1	<0.1	<0.1

TABLE 8. (CONTINUED) - METALS (MG/L) IN EFFLUENT AND DILUENT WATER.[c]

Parameter	Date of Sample			
	07/24/90	09/27/90	11/07/90	02/06/91
		Effluent		
Aluminum	0.21	0.23		
Boron	0.32	0.24	0.071	0.28
Calcium	46	46	42	46
Iron	2.27	2.0	2.6	2.4
Lead		0.005	0.006	
Magnesium	5.91	6.3	6.3	6.4
Manganese	0.59	0.74	0.667	1.2
Potassium	16.2	25.0	12.0	11.4
Sodium	29.4	37.8	39.7	39.9
		Diluent Water		
Boron			0.259	
Calcium	25	20	21	17
Iron	0.15			
Magnesium	3.97	5.1	5.4	4.6
Potassium	2.8	2.3	2.2	1.6
Sodium	5.7	7.2	7.0	7.3

TABLE 8. CONTINUED - VOLATILE AND SEMI-VOLATILE ORGANICS (MICROGRAMS/L) IN EFFLUENT.[c,d]

Parameter	Date of Sample			
	07/24/90	09/27/90	11/07/90	02/06/91
		Volatiles in Effluent		
Benzene				48.0
Chloroform		11.0	5.0	
Ethylbenzene				10.0
Total xylenes				44.0
Toluene				14.0
		Semi-volatiles in Effluent		
Bis(2-ethylhexyl)phthalate		11.0		160.0
Phenol				54.0

TABLE 8. (CONTINUED) - PESTICIDE/PCBs AND HERBICIDES (MICROGRAMS/L) IN EFFLUENT AND DILUENT WATER.[c,e]

Parameter	Date of Sample			
	07/24/90	09/27/90	11/07/90	02/06/91
		Pesticides/PCBs in Effluent		
Dieldrin	0.02			
Endosulfan sulfate	0.02			
Endrin	0.04			
Methoxychlor	0.19			
		Pesticides/PCBs in Diluent Water		
Aldrin		0.025		
		Herbicides in Effluent		
2,4-D				2.5

[a] Concentration is mg/L for all parameters except conductivity which is umho/cm.
[b] Analysis not conducted.
[c] Only compounds detected at or above the quantitation limits in Table 3 are reported.
[d] No volatiles or semi-volatiles were found in the diluent water.
[e] No herbicides were found in the diluent water.

TABLE 9. MEAN WATER QUALITY OF EACH TREATMENT TANK IN CARCINOGENICITY TEST (T) - DILUENT WATER.

Tank No.	Tank Conc	DEN Conc (mg/L)	Statistical Parameters	Temp. (°C)	pH	DO (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
01	Diluent Water	0.0	Mean	25.1	7.50	7.9	41	94
			S.D.	0.79	0.144	0.27	2.4	8.5
			Min	21.8	7.13	7.0	34	86
			Max	29.0	7.98	8.4	48	103
			N	167	167	167	23	23
02	Diluent Water	10.0	Mean	25.0	7.49	7.9	42	92
			S.D.	0.77	0.144	0.25	9.9	8.3
			Min	22.1	7.12	7.2	27	85.5
			Max	28.8	7.91	8.4	85	103
			N	167	167	167	23	23
03	Diluent Water	0.0	Mean	24.8	7.51	8.0	41	95
			S.D.	0.78	0.152	0.26	2.4	12.2
			Min	22.3	7.12	7.1	34	68
			Max	28.2	7.98	8.4	48	120
			N	167	167	167	23	23
04	Diluent Water	10.0	Mean	24.8	7.51	8.0	42	92
			S.D.	0.79	0.174	0.26	9.2	8.2
			Min	22.6	7.09	7.1	27	86
			Max	28.2	8.07	8.4	85	103
			N	176	176	176	25	25

TABLE 9. (CONTINUED) - DILUENT WATER CON'T.

Tank No.	Tank Conc	DEN Conc (mg/L)	Statistical Parameters	Chlorine		Ammonia-Nitrogen	
				Total Residual (mg/L)	Free Available (mg/L)	Total (mg/L)	Total Un-ionized (mg/L)
01	Diluent Water	0.0	Mean	0.00	0.00	0.006	0.00011
			S.D.	0.000	0.000	0.0117	0.000213
			Min	0.00	0.00	0.000	0.00000
			Max	<0.05	0.00	0.039	0.00071
			N	22	23	10	10
02	Diluent Water	10.0	Mean	0.00	0.00	0.010	0.00039
			S.D.	0.000	0.000	0.0155	0.000726
			Min	0.00	0.00	0.000	0.00000
			Max	<0.03	0.00	0.050	0.00240
			N	22	23	10	10
03	Diluent Water	0.0	Mean	0.00	0.00	0.005	0.00009
			S.D.	0.000	0.000	0.0080	0.000147
			Min	0.00	0.00	0.000	0.00000
			Max	<0.05	0.00	0.025	0.00046
			N	22	23	10	10
04	Diluent Water	10.0	Mean	0.00	0.00	0.007	0.00012
			S.D.	0.000	0.000	0.0114	0.000212
			Min	0.00	0.00	0.000	0.00000
			Max	<0.03	0.00	0.037	0.00070
			N	25	26	10	10

TABLE 9. (CONTINUED) - 10% EFFLUENT.

Tank No.	Tank Conc	DEN Conc (mg/L)	Statistical Parameters	Temp. (°C)	pH	DO (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
05	10% Effluent	0.0	Mean	24.7	7.27	7.3	41	101
			S.D.	0.75	0.134	0.46	2.0	14.2
			Min	22.9	6.81	5.4	34	86
			Max	28.2	7.57	8.4	48	137
			N	167	167	167	23	23
06	10% Effluent	10.0	Mean	24.8	7.24	7.2	43	98
			S.D.	0.78	0.122	0.54	9.7	12.6
			Min	23.0	6.77	5.3	27	68
			Max	28.4	7.56	8.4	85	120
			N	167	167	167	23	23
07	10% Effluent	0.0	Mean	24.8	7.25	7.2	41	103
			S.D.	0.78	0.126	0.54	1.4	12.4
			Min	22.9	6.77	5.3	41	86
			Max	28.4	7.56	8.4	48	120
			N	167	167	167	23	23
08	10% Effluent	10.0	Mean	24.9	7.26	7.2	42	102
			S.D.	0.78	0.131	0.54	9.8	18.5
			Min	23.1	6.80	5.4	27	86
			Max	28.4	7.57	8.4	85	171
			N	167	167	167	23	23

TABLE 9. (CONTINUED) - 10% EFFLUENT CON'T.

Tank No.	Tank Conc	DEN Conc (mg/L)	Statistical Parameters	Chlorine Total Residual (mg/L)	Chlorine Free Available (mg/L)	Ammonia-Nitrogen Total (mg/L)	Ammonia-Nitrogen Total Un-ionized (mg/L)
05	10% Effluent	0.0	Mean	0.04	0.00	0.587	0.00530
			S.D.	0.021	0.004	0.1976	0.002165
			Min	0.00	0.00	0.286	0.00223
			Max	<0.10	0.01	0.844	0.01019
			N	9	17	10	10
06	10% Effluent	10.0	Mean	0.04	0.00	0.540	0.00450
			S.D.	0.029	0.000	0.2379	0.001913
			Min	0.00	0.00	0.205	0.00149
			Max	0.08	<0.01	0.979	0.00799
			N	12	18	10	10
07	10% Effluent	0.0	Mean	0.04	0.00	0.522	0.00487
			S.D.	0.020	0.003	0.2011	0.001838
			Min	0.00	0.00	0.303	0.00231
			Max	<0.10	0.01	0.898	0.00831
			N	10	17	10	10
08	10% Effluent	10.0	Mean	0.03	0.00	0.467	0.00407
			S.D.	0.026	0.005	0.2374	0.001866
			Min	0.00	0.00	0.210	0.00174
			Max	0.07	0.02	0.958	0.00866
			N	12	18	10	10

TABLE 9. (CONTINUED) - 100% EFFLUENT.

Tank No.	Tank Conc	DEN Conc (mg/L)	Statistical Parameters	Temp. (°C)	pH	DO (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
09	100% Effluent	0.0	Mean	24.9	7.15	5.5	90	173
			S.D.	0.83	0.139	1.11	26.1	23.8
			Min	22.8	6.81	3.0	61	137
			Max	28.2	7.50	7.8	153	222
			N	167	167	167	23	23
10	100% Effluent	10.0	Mean	24.7	7.13	5.4	87	167
			S.D.	0.87	0.134	1.12	22.1	19.0
			Min	22.6	6.60	3.2	54	137
			Max	28.2	7.46	7.8	136	205
			N	167	167	167	23	23
11	100% Effluent	0.0	Mean	24.7	7.19	5.7	87	161
			S.D.	0.75	0.169	1.10	22.0	26.5
			Min	22.7	6.74	2.9	54	68
			Max	27.5	7.58	7.9	136	205
			N	176	176	176	25	25
12	100% Effluent	10.0	Mean	24.7	7.23	5.9	95	172
			S.D.	0.78	0.169	1.18	25.1	24.9
			Min	22.9	6.83	2.6	61	120
			Max	28.0	7.66	8.1	170	222
			N	167	167	167	23	23

TABLE 9. (CONTINUED) - 100% EFFLUENT CON'T.

Tank No.	Tank Conc	DEN Conc (mg/L)	Statistical Parameters	Chlorine Total Residual (mg/L)	Chlorine Free Available (mg/L)	Ammonia-Nitrogen Total (mg/L)	Ammonia-Nitrogen Total Un-ionized (mg/L)
09	100% Effluent	0.0	Mean	0.08	0.02	9.847	0.10274
			S.D.	0.036	0.006	3.1900	0.046429
			Min	<0.01	0.00	4.320	0.01858
			Max	0.20	0.03	13.700	0.16196
			N	21	19	10	10
10	100% Effluent	10.0	Mean	0.06	0.01	8.193	0.07523
			S.D.	0.034	0.009	4.8819	0.064270
			Min	0.00	0.00	3.565	0.02014
			Max	0.15	0.02	15.900	0.20239
			N	21	14	10	10
11	100% Effluent	0.0	Mean	0.11	0.03	9.777	0.11420
			S.D.	0.109	0.034	3.7803	0.098563
			Min	0.00	0.00	3.375	0.02292
			Max	0.40	0.12	16.150	0.31654
			N	25	20	10	10
12	100% Effluent	10.0	Mean	0.07	0.02	10.460	0.12637
			S.D.	0.037	0.006	2.8668	0.071285
			Min	<0.01	0.00	6.550	0.01434
			Max	0.20	0.03	15.000	0.26505
			N	21	18	10	10

APPENDIX 1

ROTIFER 24-H ACUTE TEST

Test Method:	Rotifer ToxKit™ Screening Test (US TOXKIT, Tampa, FL)
Type of Test:	Static
Date:	July 25-27, 1990
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Rotifer ToxKit™ synthetic medium
Test Organism:	
Scientific Name:	<u>Brachionus</u> <u>rubens</u>
Wet Weight:	n/a
Length:	n/a
Age:	<4 h after hatch
Source:	Rotifer ToxKit™ cyst
Experimental Chambers:	
Material:	Glass Petri dish
Volume:	10 mL
No. Organisms Per Treatment:	10
Loading:	n/a
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	No aeration during test
Endpoint:	Mortality

Endpoint:	Mortality
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.5 mg/L (Range 7.2-7.7) APHA Standard Methods (1989)
pH:	7.6 (Range 7.3-7.8) APHA Standard Methods (1989)
Conductivity:	342 umhos/cm (Range 325-360) APHA Standard Methods (1989)
Alkalinity:	103 mg/L as $CaCO_3$ (Range 90-120) APHA Standard Methods (1989)
Hardness:	235 mg/L as $CaCO_3$ (Range 176-270) APHA Standard Methods (1989)
Temperature:	25 ± 0.5°C

Results: The effluent did not affect survival. The data are summarized in Table A1-1.

TABLE A1-1. SURVIVAL OF ROTIFERS AFTER 24 HOURS EXPOSURE TO APG-WWTP EFFLUENT.

Parameter	Rep	Number Tested	No. Alive at End of Test	Percent Alive
Growth	A	10	10	100
Medium	B	10	9	90
	C	10	10	100
APG	A	10	8	80
Diluent	B	10	10	100
Water	C	10	9	90
100%	A	10	10	100
Effluent	B	10	9	90
	C	10	9	90

Results: No difference in survival occurred between organisms in ToxKit™ synthetic medium, APG diluent water, or 100% effluent. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Rotifer Survival

Data Transformation:

Arc-sine square-root transformation was used for dealing with values of 0 and 1.0 (Horning and Weber, 1985).

Chi-Square Test for Normality:

Calculated test statistic:	5.98
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.55
Alpha value:	0.01
Critical value:	9.21
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.57
Alpha value:	0.05
Critical value:	5.14
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 2

ROTIFER 24-H ACUTE TEST

Test Method:	Rotifer ToxKit™ Screening Test (US TOXKIT, Tampa, FL)
Type of Test:	Static
Date:	November 10-12, 1990
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Rotifer ToxKit™ synthetic medium
Test Organism:	
Scientific Name:	*Brachionus rubens*
Wet Weight:	n/a
Length:	n/a
Age:	<4 h after hatch
Source:	Rotifer ToxKit™ cyst
Experimental Chambers:	
Material:	Glass Petri dish
Volume:	10 mL
No. Organisms Per Treatment:	10
Loading:	n/a
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	No aeration during test
Endpoint:	Mortality

Endpoint:	Mortality
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.5 mg/L (Range 7.3-7.6) APHA Standard Methods (1989)
pH:	7.4 (Range 7.0-7.6) APHA Standard Methods (1989)
Conductivity:	330 umhos/cm (Range 274-375) APHA Standard Methods (1989)
Alkalinity:	103 mg/L as $CaCO_3$ (Range 90-120) APHA Standard Methods (1989)
Hardness:	184 mg/L as $CaCO_3$ (Range 168-191) APHA Standard Methods (1989)
Temperature:	25 ± 0.5°C

Results: The effluent did not affect survival. The data are summarized in Table A2-1.

TABLE A2-1. SURVIVAL OF ROTIFERS AFTER 24 HOURS EXPOSURE TO APG-WWTP EFFLUENT.

Parameter	Rep	Number Tested	No. Alive at End of Test	Percent Alive
Growth Medium	A	10	9	90
	B	10	10	100
	C	10	10	100
APG Diluent Water	A	10	10	100
	B	10	8	80
	C	10	10	100
100% Effluent	A	10	10	100
	B	10	10	100
	C	10	7	70

Results: No difference in survival occurred between organisms in ToxKit™ synthetic medium, APG diluent water, or 100% effluent. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Rotifer Survival

Data Transformation:

Arc-sine square-root transformation was used for dealing with values of 0 and 1.0 (Horning and Weber, 1985).

Chi-Square Test for Normality:

Calculated test statistic:	11.66
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.30
Alpha value:	0.01
Critical value:	9.21
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.18
Alpha value:	0.05
Critical value:	5.14
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 3

ROTIFER 24-H ACUTE TEST

Test Method:	Rotifer ToxKit™ Screening Test (US TOXKIT, Tampa, FL)
Type of Test:	Static
Date:	February 8-10, 1991
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Rotifer ToxKit™ synthetic medium
Test Organism:	
Scientific Name:	<u>Brachionus</u> <u>rubens</u>
Wet Weight:	n/a
Length:	n/a
Age:	<4 h after hatch
Source:	Rotifer ToxKit™ cyst
Experimental Chambers:	
Material:	Glass Petri dish
Volume:	10 mL
No. Organisms Per Treatment:	10
Loading:	n/a
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	No aeration during test
Endpoint:	Mortality

Endpoint:	**Mortality**
Mean Water Chemistry Values:	
Dissolved Oxygen:	**8.1 mg/L** **(Range 8.0-8.3)** **APHA Standard Methods (1989)**
pH:	**7.7** **(Range 7.1-8.3)** **APHA Standard Methods (1989)**
Conductivity:	**345 umhos/cm** **(Range 180-520)** **APHA Standard Methods (1989)**
Alkalinity:	**83 mg/L as $CaCO_3$** **(Range 20-135)** **APHA Standard Methods (1989)**
Hardness:	**155 mg/L as $CaCO_3$** **(Range 96-200)** **APHA Standard Methods (1989)**
Temperature:	**25 ± 0.4°C**

Results: The effluent did not affect survival. The data are summarized in Table A3-1.

TABLE A3-1. SURVIVAL OF ROTIFERS AFTER 24 HOURS EXPOSURE TO APG-WWTP EFFLUENT.

Parameter	Rep	Number Tested	No. Alive at End of Test	Percent Alive
Growth Medium	A	10	9	90
	B	10	10	100
	C	10	10	100
APG Diluent Water	A	10	10	100
	B	10	10	100
	C	10	8	80
100% Effluent	A	10	9	90
	B	10	9	90
	C	10	10	100
12.5% Effluent by Volume	A	10	10	100
	B	10	9	90
	C	10	10	100

Results: No difference in survival occurred between organisms in ToxKit™ synthetic medium, APG diluent water, 100% effluent, or 12.5% effluent by volume. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Rotifer Survival

Data Transformation:

Arc-sine square-root transformation was used for dealing with values of 0 and 1.0 (Horning and Weber, 1985).

Shapiro-Wilks Test for Normality:

Calculated test statistic:	0.88
Alpha value:	0.01
Critical value:	0.81
Conclusion:	Fail to rejected the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	1.14
Alpha value:	0.01
Critical value:	11.34
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.21
Alpha value:	0.05
Critical value:	4.07
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 4

GREEN ALGAL 96-H GROWTH TEST

Test Method:	Horning and Weber (1985)
Type of Test:	Static
Date:	July 24-28, 1990
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Double strength "AAP" medium (Miller et al., 1978) with P added to achieve a 20:1 N:P atomic ratio.
Test Organism:	
Scientific Name:	Selenastrum capricornutum
Age:	Log growth
Source:	University of Texas culture collection
Experimental Chambers:	
Material:	Glass culture flasks with cheesecloth/cotton stoppers
Volume:	500 mL
Initial Cell Density:	≈5 x 10^3 cells/mL
Lighting:	Fluorescent; cool white; continuous; ≈300 foot candles
Aeration:	None

Endpoint:	**Reduction in growth rate relative to control**
Temperature:	**20 ± 0.5°C**

Results: The effluent did not affect growth rate. The data are summarized in Tables A4-1 and A4-2.

TABLE A4-1. MEAN CELL DENSITY (CELLS/ML) OF GREEN ALGA EXPOSED TO APG-WWTP EFFLUENT.

Conc (Percent Effluent by Vol)	Rep	Mean Cell Density				
		0H	24H	48H	72H	96H
Growth Medium	1	3350	54770	168100	331905	734220
	2	3577	51630	169260	320700	729440
	3	2826	59600	166660	319710	731260
APG Diluent Water	1	3331	52180	165220	320330	734160
	2	3678	56010	165990	315570	730230
	3[a]					
6.25	1	2917	56100	168230	316190	740840
	2	2874	52850	170300	315670	743180
	3	2529	60870	168490	316840	734570
12.5	1	2652	54420	171900	349490	744530
	2	2834	59130	163410	373680	745270
	3	3044	61120	169720	316620	744730
25.0	1	2100	60690	171420	328090	739950
	2	2540	66830	175300	350290	740690
	3	3262	70290	173580	371890	738350
50.0	1	2684	75120	173820	384300	752370
	2	3094	66000	178080	366330	763290
	3	2328	68720	180150	391930	765650
99.0	1	3372	67930	173470	315940	743290
	2	3057	68300	166250	330410	748000
	3	2373	65730	167100	336520	742550

[a] Lost sample.

TABLE A4-2. GROWTH RATE OF GREEN ALGA AFTER 96 HOURS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Growth Rate Per Day[a]	Mean Growth Rate Per Day	Relative Growth Rate
Growth Medium	1	0.585		
	2	0.577		
	3	0.603	0.588	100.0
APG Diluent Water	1	0.586		
	2	0.574		
	3[b]		0.580	98.6
6.25	1	0.601		
	2	0.603		
	3	0.616	0.607	103.1
12.5	1	0.612		
	2	0.605		
	3	0.597	0.605	102.8
25.0	1	0.637		
	2	0.616		
	3	0.589	0.614	104.4
50.0	1	0.612		
	2	0.598		
	3	0.629	0.613	104.2
99.0	1	0.586		
	2	0.597		
	3	0.624	0.602	102.4

[a] Growth Rate = $\log_{10}n_1 - \log_{10}n_2 / t_1 - t_2$, where

n_1 = cell density (cells/mL) at day 4

n_2 = cell density (cells/mL) at day 0

t = time in days.

[b] Lost sample.

APPENDIX 5

GREEN ALGAL 96-H GROWTH TEST

Test Method:	Horning and Weber (1985)
Type of Test:	Static
Date:	November 8-12, 1990
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Double strength "AAP" medium (Miller et al., 1978) with P added to achieve a 20:1 N:P atomic ratio
Test Organism:	
Scientific Name:	*Selenastrum capricornutum*
Age:	Log growth
Source:	University of Texas culture collection
Experimental Chambers:	
Material:	Glass culture flasks with cheesecloth/cotton stoppers
Volume:	500 mL
Initial Cell Density:	≈5 x 10^3 cells/mL
Lighting:	Fluorescent; cool white; continuous; ≈300 foot candles
Aeration:	None

Endpoint:	Reduction in growth rate relative to control
Temperature:	20 ± 0.3°C

Results: The effluent did not affect growth rate. The data are summarized in Tables A5-1 and A5-2.

TABLE A5-1. MEAN CELL DENSITY (CELLS/ML) OF GREEN ALGA EXPOSED TO APG-WWTP EFFLUENT.

Conc (Percent Effluent by Vol)	Rep	Mean Cell Density				
		0H	24H	48H	72H	96H
Growth Medium	1	6583	64370	240645	684675	1170384
	2	6788	71953	233910	681840	1169280
	3	6413	70453	244590	685200	1168692
APG Diluent Water	1	6665	58103	232995	683955	1162608
	2	6839	57507	229485	682740	1156008
	3	6573	57753	237480	685455	1105200
6.25	1	6486	61930	235800	685290	1106304
	2	6437	75933	226935	683490	1070400
	3	6265	72173	235360	683445	1103328
12.5	1	6326	73387	236115	683400	1126776
	2	6417	71810	237795	675660	1120824
	3	6522	74776	238905	683475	1118688
25.0	1	6050	74447	234720	683640	1101312
	2	6270	73477	251730	680850	1239768
	3	6631	74980	235695	682050	1179840
50.0	1	6342	75113	244215	682845	1164648
	2	6547	76520	239340	687465	1228272
	3	6164	74983	236925	684525	1144056
99.0	1	6686	75457	237360	689145	1172424
	2	6529	72267	239145	716850	1191408
	3	6187	75603	263310	706545	1163688

TABLE A5-2. GROWTH RATE OF GREEN ALGA AFTER 96 HOURS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Growth Rate Per Day[a]	Mean Growth Rate Per Day	Relative Growth Rate
Growth Medium	1	0.562		
	2	0.559		
	3	0.565	0.552	100.0
APG Diluent Water	1	0.560		
	2	0.557		
	3	0.556	0.558	99.3
6.25	1	0.558		
	2	0.555		
	3	0.561	0.558	99.3
12.5	1	0.563		
	2	0.561		
	3	0.559	0.561	99.8
25.0	1	0.565		
	2	0.574		
	3	0.563	0.567	100.9
50.0	1	0.566		
	2	0.568		
	3	0.567	0.567	100.9
99.0	1	0.561		
	2	0.565		
	3	0.569	0.565	100.5

[a] Growth Rate = $\log_{10}n_1 - \log_{10}n_2 / t_1 - t_2$, where

n_1 = cell density (cells/mL) at day 4

n_2 = cell density (cells/mL) at day 0

t = time in days.

APPENDIX 6

GREEN ALGAL 96-H GROWTH TEST

Test Method:	Horning and Weber (1985)
Type of Test:	Static
Date:	February 12-16, 1991
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Test Medium:	Double strength "AAP" medium (Miller et al., 1978) with P added to achieve a 20:1 N:P atomic ratio
Test Organism:	
Scientific Name:	Selenastrum capricornutum
Age:	Log growth
Source:	University of Texas culture collection
Experimental Chambers:	
Material:	Glass culture flasks with cheesecloth/cotton stoppers
Volume:	500 mL
Initial Cell Density:	5×10^3 cells/mL
Lighting:	Fluorescent; cool white; continuous; ≈300 foot candles
Aeration:	None

Endpoint:	Reduction in growth rate (relative to control)
Temperature:	20 ± 0.4°C

Results: Significant reductions in growth relative to the control organisms occurred in the APG diluent water and 100% effluent. The EC50, NOEC, and LOEC for the effluent are as follows:

96-h EC50: Could not be estimated by the probit statistic because a significant reduction in growth occurred in only one concentration.

NOEC: 50% effluent by volume.

LOEC: 99% effluent by volume.

See Tables A6-1, A6-2, and A6-3 for additional data.

TABLE A6-1. MEAN CELL DENSITY (CELLS/ML) OF GREEN ALGA EXPOSED TO APG-WWTP EFFLUENT.

Conc (Percent Effluent by Vol)	Rep	Mean Cell Density				
		0H	24H	48H	72H	96H
Growth Medium	1	2113	29613	242630	566210	1043960
	2	2835	29235	288490	558970	1004290
	3	2630	28875	251040	560980	998860
APG Diluent Water	1	2525	30005	207860	475830	942630
	2	2620	28130	224390	470750	948360
	3	2670	29400	216740	521190	960940
6.25	1	2570	30585	218760	512020	1004970
	2	2303	30190	294990	518770	966620
	3	2628	29265	277590	543860	950500
12.5	1	3100	28750	278830	591970	990580
	2	3058	30205	248460	585670	965160
	3	2980	30980	249340	600180	1001730
25.0	1	3170	32268	301430	542070	955600
	2	2945	32940	241040	541040	1001410
	3	2885	35013	279880	569100	998470
50.0	1	3155	29900	279860	566840	1024750
	2	3055	31640	315430	493980	962060
	3	2720	30728	284300	512800	969860
99.0	1	2875	28542	158410	206490	340940
	2	2753	27983	158120	198900	302110
	3	2568	28038	129460	201580	288010

TABLE A6-2. GROWTH RATE OF GREEN ALGA AFTER 96 HOURS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Growth Rate Per Day[a]	Mean Growth Rate Per Day	Relative Growth Rate[b]
Growth Medium	1	0.673		
	2	0.637		
	3	0.645	0.651	100.0
APG Diluent Water	1	0.643		
	2	0.640		
	3	0.639	0.641	93.6
6.25	1	0.648		
	2	0.656		
	3	0.640	0.648	95.9
12.5	1	0.626		
	2	0.625		
	3	0.632	0.628	96.6
25.0	1	0.620		
	2	0.633		
	3	0.635	0.629	97.0
50.0	1	0.628		
	2	0.625		
	3	0.638	0.630	97.0
99.0	1	0.519		
	2	0.510		
	3	0.512	0.514	30.6

[a] Growth Rate = $\log_{10}n_1 - \log_{10}n_2 / t_1 - t_2$, where

n_1 = cell density (cells/mL) at day 4

n_2 = cell density (cells/mL) at day 0

t = time in days.

[b] Relative growth rate at 96 h derived from the arithmetic means at each treatment.

Statistical Analysis of Algal Cell Growth for NOEC and LOEC

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	5.72
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	9.33
Alpha value:	0.01
Critical value:	16.81
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	284.18
Alpha value:	0.05
Critical value:	2.85
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistic:	See Table A6-3
Alpha value:	0.05
Critical value:	2.53
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A6-3. RESULTS OF DUNNETT'S TEST ON MEAN CELL DENSITY (CELLS/ML) OF GREEN ALGA AFTER 96 HOURS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by volume)	No. of Reps	Mean Cell Density	T Statistic	Significance
Growth Medium	3	1,015,703		
APG Diluent Water	3	950,643	3.05	a
6.25	3	974,030	1.95	
12.5	3	980,823	1.63	
25	3	985,160	1.43	
50	3	985,556	1.41	
99	3	310,353	33.03	a

[a] Significantly different at alpha = 0.05 (Dunnett critical value = 2.53).

APPENDIX 7

CLADOCERAN 7-D SURVIVAL AND REPRODUCTION TEST

Test Method:	Waller and Lazorchak (1986)
Type of Test:	Static renewal (every 24 h)
Date:	July 24-31, 1990
Investigators:	S. D. Turley E. P. Smithers
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Ceriodaphnia dubia*
Wet Weight:	n/a
Length:	n/a
Age:	<12 h
Source:	JHU/APL-AES Culture
Experimental Chambers:	
Material:	50 mL glass beakers
Volume:	30 mL
No. Organisms Per Treatment:	10
Loading:	1 organism/beaker
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a

Aeration:	Prior to each renewal
Endpoints:	Mortality of adults; number of neonates produced in 3 broods
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.2 mg/L (Range 6.8-7.7) APHA Standard Methods (1989)
pH:	7.8 (Range 7.2-8.3) APHA Standard Methods (1989)
Conductivity:	344 umhos/cm (Range 310-380) APHA Standard Methods (1989)
Alkalinity:	106 mg/L as $CaCO_3$ (Range 80-130) APHA Standard Methods (1989)
Hardness:	228 mg/L as $CaCO_3$ (Range 172-278) APHA Standard Methods (1989)
Mean Temperatures:	25°C (Range 24.5-25.5)

Results: The effluent did not affect the survival of the adults or the production of neonates. The data are summarized in Tables A7-1, A7-2, and A7-3.

TABLE A7-1. SURVIVAL OF DAPHNID ADULTS AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES Diluent Water	10	10[a]	100
APG Diluent Water	10	10[a]	100
6.25	10	10[a]	100
12.5	10	10[b]	100
25.0	10	9	90
50.0	10	10	100
100	10	10[b]	100

[a] Two adult males were included in the counts; therefore, only eight daphnids produced broods.

[b] One adult male was included in the counts; therefore, only nine daphnids produced broods.

Results: The effluent did not affect the survival of the adults.

TABLE A7-2. SUMMARY OF LIVING DAPHNID OFFSPRING PRODUCED AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	N	Mean Number	Range
JHU/APL-AES Diluent Water	8	29.4	28 - 32
APG Diluent Water	8	28.6	26 - 32
6.25	8	29.8	27 - 32
12.5	9	30.9	27 - 37
25.0	9	32.6	29 - 35
50.0	10	31.9	26 - 39
100	9	29.1	27 - 32

TABLE A7-3. NUMBER OF DAPHNID YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD.

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Total No. 3	Total Young	Mean Young Per Brood
JHU/APL-AES Diluent Water	1	4	9	16	29	9.7
	2	4	9	15	28	9.3
	3	4	10	17	31	10.3
	4	5	13	14	32	10.7
	5	4	11	13	28	9.3
	6	4	10	15	29	9.7
	7	4	11	15	30	10.0
	8	5	9	14	28	9.3
APG Diluent Water	1	4	13	15	32	10.7
	2	4	9	17	30	10.0
	3	4	10	14	28	9.3
	4	3	9	15	27	9.0
	5	3	9	14	26	8.7
	6	4	8	15	27	9.0
	7	3	10	16	29	9.7
	8	4	10	16	30	10.0
6.25	1	4	11	16	31	10.3
	2	5	9	13	27	9.0
	3	2	11	16	29	9.7
	4	4	8	17	29	9.7
	5	4	11	16	31	10.3
	6	6	11	15	32	10.7
	7	5	10	16	31	10.3
	8	4	9	15	28	9.3
12.5	1	4	15	12	31	10.3
	2	4	10	13	27	9.0
	3	4	11	16	31	10.3
	4	4	11	16	31	10.3
	5	4	11	14	29	9.7
	6	4	12	15	31	10.3
	7	4	12	14	30	10.0
	8	4	12	21	37	12.3
	9	5	12	14	31	10.3

TABLE A7-3. (CONTINUED).

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
25	1	5	9	18	32	10.7
	2	4	11	17	32	10.7
	3	4	13	18	35	11.7
	4	5	12	17	34	11.3
	5	5	12	17	34	11.3
	6	4	12	18	34	11.3
	7	4	10	19	33	11.0
	8	3	11	16	30	10.0
	9	5	10	14	29	9.7
	10	*				
50	1	4	10	17	31	10.3
	2	4	11	18	33	11.0
	3	4	13	18	35	11.7
	4	4	10	18	32	10.7
	5	4	13	19	36	12.0
	6	5	12	22	39	13.0
	7	4	11	20	35	11.7
	8	4	9	17	30	10.0
	9	4	9	17	30	10.0
	10	3	9	14	26	8.7
100	1	4	13	15	32	10.7
	2	3	12	14	29	9.7
	3	4	12	12	28	9.3
	4	5	8	14	27	9.0
	5	3	11	17	31	10.3
	6	4	11	15	30	10.0
	7	2	9	16	27	9.0
	8	4	13	14	31	10.3
	9	2	10	15	27	9.0

* Daphnid died prior to end of test.

Results: The effluent did not affect the total number of neonates produced. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Total Daphnid Neonates Produced Per Adult

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	1.27
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	10.78
Alpha value:	0.01
Critical value:	16.81
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.34
Alpha value:	0.05
Critical value:	3.23
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 8

CLADOCERAN 7-D SURVIVAL AND REPRODUCTION TEST

Test Method:	Waller and Lazorchak (1986)
Type of Test:	Static renewal (every 24 h)
Date:	November 5-12, 1990
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Ceriodaphnia dubia*
Wet Weight:	n/a
Length:	n/a
Age:	<12 h
Source:	JHU/APL-AES Culture
Experimental Chambers:	
Material:	50 mL glass beakers
Volume:	30 mL
No. Organisms Per Treatment:	10
Loading:	1 organism/beaker
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	Prior to each renewal

Endpoints:	Mortality of adults; number of neonates produced in 3 broods

Mean Water Chemistry Values:

Dissolved Oxygen:	6.8 mg/L (Control) 7.0 mg/L (High effluent concentration) (Range 5.9-8.2) APHA Standard Methods (1989)
pH:	7.3 (Control) 7.1 (High effluent concentration) (Range 6.4-8.1) APHA Standard Methods (1989)
Conductivity:	304 umhos/cm (Control) 369 umhos/cm (High effluent concentration) (Range 260-400) APHA Standard Methods (1989)
Alkalinity:	86 mg/L as $CaCO_3$ (Control) 119 mg/L (High effluent concentration) (Range 60-140) APHA Standard Methods (1989)
Hardness:	180 mg/l as $CaCO_3$ (Control) 195 mg/L as $CaCo_3$ (High effluent concentration) (Range 150-230) APHA Standard Methods (1989)
Mean Temperature:	25.1°C (Range 25.0-25.4)

Results: The effluent did not affect the survival of the adults or the production of neonates. The data are summarized in Tables A8-1, A8-2, and A8-3.

TABLE A8-1. SURVIVAL OF DAPHNID ADULTS AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES Diluent Water	10	10	100
APG Diluent Water	10	10	100
6.25	10	9	90
12.5	10	10	100
25.0	10	10	100
50.0	10	10	100
100	10	10	100

Results: The effluent did not affect the survival of the adults.

TABLE A8-2. SUMMARY OF LIVING DAPHNID OFFSPRING PRODUCED AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	N	Mean Number	Range
JHU/APL-AES Diluent Water	10	28.8	28 - 30
APG Diluent Water	10	27.1	25 - 30
6.25	9	27.7	26 - 31
12.5	10	27.5	24 - 33
25.0	10	27.4	26 - 31
50.0	10	28.8	24 - 33
100	10	26.4	25 - 28

TABLE A8-3. NUMBER OF DAPHNID YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD.

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
JHU/APL-AES Diluent Water	1	4	9	17	30	10.0
	2	3	9	16	28	9.3
	3	3	12	15	30	10.0
	4	3	12	14	29	9.7
	5	4	8	16	28	9.3
	6	4	9	15	28	9.3
	7	5	8	16	29	9.7
	8	4	10	16	30	10.0
	9	5	9	14	28	9.3
	10	4	8	16	28	9.3
APG Diluent Water	1	3	8	15	26	8.7
	2	3	7	18	28	9.3
	3	4	8	13	25	8.3
	4	3	8	14	25	8.3
	5	3	10	14	27	9.0
	6	3	11	16	30	10.0
	7	3	9	14	26	8.7
	8	3	8	16	27	9.0
	9	3	8	16	27	9.0
	10	4	9	17	30	10.0
6.25	1	3	9	15	27	9.0
	2	5	7	15	27	9.0
	3	4	8	14	26	8.7
	4	5	9	15	29	9.7
	5	4	10	14	28	9.3
	6	4	8	16	28	9.3
	7	4	8	14	26	8.7
	8	3	9	15	27	9.0
	9	3	11	17	31	10.3
	10	*				

TABLE A8-3. (CONTINUED).

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
12.5	1	3	7	16	26	8.7
	2	5	12	16	33	11.0
	3	4	10	14	28	9.3
	4	4	10	14	28	9.3
	5	4	9	15	28	9.3
	6	2	10	17	29	9.7
	7	3	8	13	24	8.0
	8	3	8	14	25	8.3
	9	3	8	16	27	9.0
	10	3	7	17	27	9.0
25	1	6	8	13	27	9.0
	2	4	9	14	27	9.0
	3	5	11	15	31	10.3
	4	5	9	14	28	9.3
	5	4	9	14	27	9.0
	6	4	7	16	27	9.0
	7	3	8	16	27	9.0
	8	3	9	14	26	8.7
	9	3	10	13	26	8.7
	10	3	10	15	28	9.3
50	1	5	11	17	33	11.0
	2	4	11	18	33	11.0
	3	3	9	20	32	10.7
	4	4	9	16	29	9.7
	5	3	10	15	28	9.3
	6	3	8	14	25	8.3
	7	3	9	16	28	9.3
	8	4	7	13	24	8.0
	9	4	11	13	28	9.3
	10	3	8	17	28	9.3

TABLE A8-3. (CONTINUED).

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
100	1	4	8	14	26	8.7
	2	3	9	14	26	8.7
	3	3	8	15	26	8.7
	4	4	10	14	28	9.3
	5	3	8	14	25	8.3
	6	5	8	14	27	9.0
	7	4	8	15	27	9.0
	8	3	8	16	27	9.0
	9	3	9	15	27	9.0
	10	3	10	12	25	8.3

[a] Daphnid died prior to the end of the test.

Results: The effluent did not affect the total number of neonates produced. The statistical analysis of the data is summarized on the next page.

Statistical Analysis of Total Daphnid Neonates Produced Per Adult

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	4.62
Alpha value:	0.01
Critical value:	4.89
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Hartley Test for Homogeneity of Variances:

Calculated test statistic:	11.26
Alpha value:	0.01
Critical value:	13.10
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.13
Alpha value:	0.05
Critical value:	2.25
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 9

CLADOCERAN 7-D SURVIVAL AND REPRODUCTION TEST

Test Method:	Waller and Lazorchak (1986)
Type of Test:	Static renewal (every 24 h)
Date:	February 6-13, 1991
Investigator:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Ceriodaphnia dubia*
Wet Weight:	n/a
Length:	n/a
Age:	<12 h
Source:	JHU/APL-AES Culture
Experimental Chambers:	
Material:	50 mL glass beakers
Volume:	30 mL
No. Organisms Per Treatment:	10
Loading:	1 organism/beaker
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	Prior to each renewal

Endpoints:	Mortality of adults; number of neonates produced in 3 broods
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.8 mg/L (Control) 7.7 mg/L (High effluent concentration) (Range 7.3-8.2) APHA Standard Methods (1989)
pH:	8.0 (Control) 7.3 (High effluent concentration) (Range 7.1-8.3) APHA Standard Methods (1989)
Conductivity:	336 umhos/cm (Control) 510 umhos/cm (High effluent concentration) (Range 330-520) APHA Standard Methods (1989)
Alkalinity:	141 mg/L as $CaCO_3$ (Control) 96 mg/L (High effluent concentration) (Range 90-150) APHA Standard Methods (1989)
Hardness:	202 mg/L as $CaCO_3$ (Control) 176 mg/L as $CaCo_3$ (High effluent concentration) (Range 170-210) APHA Standard Methods (1989)
Mean Temperature:	25.3°C (Range 25.2-25.4)

Results: The effluent did not affect the survival of the adults after 7 d of exposure. A statistically significant (alpha = 0.05) increase in neonate production occurred in 100% effluent only. See Tables A9-1, A9-2, A9-3, and A9-4 for additional data.

TABLE A9-1. SURVIVAL OF DAPHNID ADULTS AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES Diluent Water	10	10	100
APG Diluent Water	10	10	100
6.25	10	10	100
12.5	10	10	100
25.0	10	10	100
50.0	10	10	100
100	10	10	100

TABLE A9-2. SUMMARY OF LIVING DAPHNID OFFSPRING PRODUCED AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	N	Mean Number	Range
JHU/APL-AES Diluent Water	10	27.9	26 - 29
APG Diluent Water	10	26.7	25 - 29
6.25	10	28.1	25 - 31
12.5	10	27.2	25 - 30
25.0	10	29.6	26 - 31
50.0	10	29.0	27 - 31
100	10	29.8	27 - 33

TABLE A9-3. NUMBER OF YOUNG PRODUCED PER BROOD, TOTAL NUMBER OF YOUNG, AND MEAN NUMBER OF YOUNG PER BROOD.

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
JHU/APL-AES Diluent Water	1	5	9	15	29	9.7
	2	4	10	15	29	9.7
	3	5	8	15	28	9.3
	4	3	9	14	26	8.7
	5	3	8	15	26	8.7
	6	4	10	14	28	9.3
	7	4	9	15	28	9.3
	8	4	8	15	27	9.0
	9	4	8	17	29	9.7
	10	4	9	16	29	9.7
APG Diluent Water	1	3	8	15	26	8.7
	2	3	10	16	29	9.7
	3	4	8	14	26	8.7
	4	3	11	14	28	9.3
	5	4	8	13	25	8.3
	6	3	8	14	25	8.3
	7	4	8	17	29	9.7
	8	3	10	15	28	9.3
	9	3	8	14	25	8.3
	10	3	7	16	26	8.7
6.25	1	4	10	17	31	10.3
	2	4	8	14	26	8.7
	3	4	10	16	30	10.0
	4	3	7	15	25	8.3
	5	4	10	15	29	9.7
	6	3	8	16	27	9.0
	7	4	10	17	31	10.3
	8	3	9	15	27	9.0
	9	4	10	13	27	9.0
	10	4	9	15	28	9.3

TABLE A9-3. (CONTINUED).

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
12.5	1	3	10	14	27	9.0
	2	3	9	16	28	9.3
	3	3	10	14	27	9.0
	4	3	10	15	28	9.3
	5	3	8	14	25	8.3
	6	3	7	15	25	8.3
	7	3	8	16	27	9.0
	8	4	9	17	30	10.0
	9	3	8	15	26	8.7
	10	4	9	16	29	9.7
25	1	2	11	14	27	9.0
	2	4	12	15	31	10.3
	3	4	10	17	31	10.3
	4	5	8	13	26	8.7
	5	4	13	17	34	11.3
	6	4	8	17	29	9.7
	7	5	11	14	30	10.0
	8	4	12	15	31	10.3
	9	3	8	15	26	8.7
	10	4	9	18	31	10.3
50	1	2	11	17	30	10.0
	2	4	8	15	27	9.0
	3	3	9	17	29	9.7
	4	4	11	16	31	10.3
	5	3	10	17	30	10.0
	6	4	9	16	29	9.7
	7	3	8	17	28	9.3
	8	3	9	16	28	9.3
	9	3	9	16	28	9.3
	10	4	9	17	30	10.0

TABLE A9-3. (CONTINUED).

Concentration (% Effluent by Volume)	Rep	Brood No. 1	Brood No. 2	Brood No. 3	Total Young	Mean Young Per Brood
100	1	4	7	16	27	9.0
	2	4	10	15	29	9.7
	3	3	9	17	29	9.7
	4	4	8	18	30	10.0
	5	4	10	19	33	11.0
	6	3	9	19	31	10.3
	7	4	11	15	30	10.0
	8	4	9	16	29	9.7
	9	3	8	18	29	9.7
	10	4	10	17	31	10.3

Statistical Analysis of Total Daphnid Neonates Produced Per Adult

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	1.77
Alpha value:	0.01
Critical value:	13.27
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Hartley Test for Homogeneity of Variances:

Calculated test statistic:	4.68
Alpha value:	0.01
Critical value:	13.10
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	4.46
Alpha value:	0.05
Critical value:	2.25
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistic:	See Table A9-4
Alpha value:	0.05
Critical value:	2.35
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A9-4. RESULTS OF DUNNETT'S TEST ON MEAN DAPHNID NEONATES PRODUCED AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by volume)	No. of Reps	Mean Neonates Produced	T Statistic	Significance
JHU/APL-AES Diluent Water	10	27.9		
APG Diluent Water	10	26.7	1.52	
6.25	10	28.1	0.25	
12.5	10	27.2	0.88	
25	10	29.6	2.15	
50	10	29.0	1.39	
100	10	29.8	2.40	*

* Significantly different at alpha = 0.05 (Dunnett critical value = 2.35).

APPENDIX 10

FATHEAD MINNOW 7-D SURVIVAL AND GROWTH TEST

Test Method:	Weber et al. (1989)
Type of Test:	Static renewal (every 24 h)
Date:	July 24-31, 1990
Investigators:	S. D. Turley E. P. Smithers
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	<u>Pimephales promelas</u>
Age:	<24 h at start of test
Source:	JHU/APL-AES culture
Experimental Chambers:	
Material:	600 mL glass beakers
Volume:	500 mL
No. Organisms Per Replicate:	10
No. Organisms Per Treatment:	40
Loading:	<0.5 g/L
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a

Flow Rate:	n/a
Aeration:	None
Endpoints:	Mortality, growth
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.2 mg/L (Range 6.8-7.7) APHA Standard Methods (1989)
pH:	7.8 (Range 7.2-8.3) APHA Standard Methods (1989)
Conductivity:	344 umhos/cm (Range 310-380) APHA Standard Methods (1989)
Alkalinity:	106 mg/L as $CaCo_3$ (Range 80-130) APHA Standard Methods (1989)
Hardness:	228 mg/L as $CaCo_3$ (Range 172-278) APHA Standard Methods (1989)
Temperature:	25°C (Range 24.3-25.7)

Results: The effluent did not affect the survival of fathead minnow larvae. Dry weight data are not reported because the samples were lost due to a malfunction in a drying oven. The survival data are summarized in Table A10-1.

TABLE A10-1. SURVIVAL OF FATHEAD MINNOW LARVAE AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES	A	10	10	100
Diluent	B	10	9	90
Water	C	10	10	100
	D	10	9	90
APG	A	10	10	100
Diluent	B	10	9	90
Water	C	10	10	100
	D	10	10	100
6.25	A	10	10	100
	B	10	9	90
	C	10	8	80
	D	10	10	100
12.5	A	10	10	100
	B	10	10	100
	C	10	9	90
	D	10	10	100
25	A	10	10	100
	B	10	7	70
	C	10	10	100
	D	10	10	100
50	A	10	8	80
	B	10	10	100
	C	10	10	100
	D	10	9	90
100	A	10	9	90
	B	10	9	90
	C	10	10	100
	D	10	10	100

Results: The effluent did not affect the survival of the larvae. The analysis of the data is summarized on the next page.

Statistical Analysis of Fathead Minnow Larval Survival

Data Transformation:

Arc-sine square-root transformation was used for dealing with values of 0 and 1.0 (Horning and Weber, 1985).

Chi-Square Test for Normality:

Calculated test statistic:	4.71
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.57
Alpha value:	0.01
Critical value:	16.81
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.35
Alpha value:	0.05
Critical value:	2.57
Conclusion:	Fail to reject the null hypothesis that all groups are equal

APPENDIX 11

FATHEAD MINNOW 7-D SURVIVAL AND GROWTH TEST

Test Method:	Weber et al. (1989) Note: Juvenile fathead minnow (17 d old) were used because larvae <24 h old were not available.
Type of Test:	Static renewal (every 24 h)
Date:	November 5-12, 1990
Investigators:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Pimephales promelas*
Age:	17 d old at start of test
Source:	JHU/APL-AES culture
Experimental Chambers:	
Material:	600 mL glass beakers
Volume:	500 mL
No. Organisms Per Replicate:	10
No. Organisms Per Treatment:	40
Loading:	<0.5 g/L
Lighting:	Fluorescent; 60-85 foot candles

Metering System:	n/a
Flow Rate:	n/a
Aeration:	None
Endpoints:	Mortality, growth
Mean Water Chemistry Values:	
Dissolved Oxygen:	6.5 mg/L (Control) 6.2 mg/L (High effluent concentration) (Range 5.4-8.7) APHA Standard Methods (1989)
pH:	7.2 (Control) 7.1 (High effluent concentration) (Range 6.9-8.0) APHA Standard Methods (1989)
Conductivity:	308 umhos/cm (Control) 375 umhos/cm (High effluent concentration) (Range 260-400) APHA Standard Methods (1989)
Alkalinity:	87 mg/L as $CaCo_3$ (Control) 118 mg/L as $CaCo_3$ (High effluent concentration) (Range 60-150) APHA Standard Methods (1989)
Hardness:	228 mg/L as $CaCo_3$ (Range 172-278) APHA Standard Methods (1989)
Temperature:	24.7°C (Range 24.1-25.2)

Results: The effluent did not affect the survival of juvenile fathead minnow. The survival data are summarized in Table A11-1.

The effluent did affect the growth of juvenile fathead minnow (Table A11-2). Statistically significant (alpha value = 0.05) mortality occurred only in the 12.5% effluent by volume concentration (Table A11-3).

TABLE A11-1. SURVIVAL OF FATHEAD MINNOW JUVENILES AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES	A	10	8	80
Diluent	B	10	10	100
Water	C	10	9	90
	D	10	10	100
APG	A	10	9	90
Diluent	B	10	10	100
Water	C	10	9	90
	D	10	10	100
6.25	A	10	10	100
	B	10	9	90
	C	10	10	100
	D	10	8	80
12.5	A	10	8	80
	B	10	10	100
	C	10	9	90
	D	10	10	100
25	A	10	10	100
	B	10	10	100
	C	10	10	100
	D	10	8	80
50	A	10	7	70
	B	10	8	80
	C	10	9	90
	D	10	9	90
100	A	10	8	80
	B	10	8	80
	C	10	8	80
	D	10	10	100

Results: The effluent did not affect the survival of the juveniles. The analysis of the data is summarized on the next page.

Statistical Analysis of Fathead Minnow Juvenile Survival

Data Transformation:

Arc-sine square-root transformation was used for dealing with values of 0 and 1.0 (Horning and Weber, 1985).

Chi-Square Test for Normality:

Calculated test statistic:	1.89
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	0.84
Alpha value:	0.01
Critical value:	16.81
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	1.11
Alpha value:	0.05
Critical value:	2.57
Conclusion:	Fail to reject the null hypothesis that all groups are equal

TABLE A11-2. GROWTH OF FATHEAD MINNOW JUVENILES AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Dry Weight (mg)	Mean Dry Weight (mg)
JHU/APL-AES Diluent Water	A	3.3	
	B	3.0	
	C	3.7	
	D	3.4	3.4
APG Diluent Water	A	4.2	
	B	3.3	
	C	3.1	
	D	3.7	3.6
6.25	A	2.5	
	B	3.1	
	C	3.1	
	D	3.2	3.0
12.5	A	2.5	
	B	2.6	
	C	2.7	
	D	3.2	2.7
25	A	3.2	
	B	3.2	
	C	3.7	
	D	3.3	3.4
50	A	3.4	
	B	3.0	
	C	2.8	
	D	3.6	3.2
100	A	2.7	
	B	3.5	
	C	3.2	
	D	3.7	3.3

Results: The effluent did affect the growth of the juveniles. Statistically significant (alpha value = 0.05) mortality occurred only in the 12.5% effluent by volume concentration. The analysis of the data is summarized on the next page and in Table A11-3.

Statistical Analysis of Fathead Minnow Juvenile Growth

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	3.49
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Hartley Test for Homogeneity of Variances:

Calculated test statistic:	3.44
Alpha value:	0.01
Critical value:	3.7
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	2.72
Alpha value:	0.05
Critical value:	2.18
Conclusion:	Reject the null hypothesis that all groups are equal

Bonferroni T-Test:

Calculated test statistic:	See Table A11-3
Alpha value:	0.05
Critical value:	2.43
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A11-3. RESULTS OF BONFERRONI T-TEST OF FATHEAD MINNOW JUVENILE GROWTH AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Group (% Effluent by Volume)	N	Mean Dry Weight (mg)	T Statistic	Significance
JHU/APL-AES Diluent Water	4	3.4		
APG Diluent Water	4	3.6	-0.905	
6.25	4	3.0	1.609	
12.5	4	2.7	2.560	*
25	4	3.4	-0.087	
50	4	3.2	0.631	
100	4	3.3	0.197	

* Significantly different at alpha = 0.05 (Bonferroni T critical value = 2.43).

APPENDIX 12

FATHEAD MINNOW 7-D SURVIVAL AND GROWTH TEST

Test Method:	Weber et al. (1989)
Type of Test:	Static renewal (every 24 h)
Date:	February 6-13, 1991
Investigators:	S. D. Turley
Laboratory:	JHU/APL-AES
Effluent:	
Source:	APG-WWTP
Chemical Characteristics:	Effluent not analyzed during test; however, see Tables 3 and 8 in text
Dilution Water:	
Source:	JHU/APL-AES deep well
Chemical Characteristics:	See Table 2 in text
Test Organism:	
Scientific Name:	*Pimephales promelas*
Age:	21 h old at start of test
Source:	JHU/APL-AES culture
Experimental Chambers:	
Material:	600 mL glass beakers
Volume:	500 mL
No. Organisms Per Replicate:	10
No. Organisms Per Treatment:	40
Loading:	<0.5 g/L
Lighting:	Fluorescent; 60-85 foot candles
Metering System:	n/a
Flow Rate:	n/a
Aeration:	None

Endpoints:	Mortality, growth
Mean Water Chemistry Values:	
Dissolved Oxygen:	7.3 mg/L (Control) 7.0 mg/L (High effluent concentration) (Range 5.9-8.6) APHA Standard Methods (1989)
pH:	7.9 (Control) 7.3 (High effluent concentration) (Range 7.2-8.3) APHA Standard Methods (1989)
Conductivity:	319 umhos/cm (Control) 524 umhos/cm (High effluent concentration) (Range 300-560) APHA Standard Methods (1989)
Alkalinity:	133 mg/L as $CaCo_3$ (Control) 104 mg/L as $CaCo_3$ (High effluent concentration) (Range 90-150) APHA Standard Methods (1989)
Hardness:	213 mg/L as $CaCo_3$ (Control) 169 mg/L as $CaCo_3$ (High effluent concentration) (Range 190-186) APHA Standard Methods (1989)
Temperature:	25.3°C (Range 25.2-25.4)

Results: The effluent affected the survival of larval fathead minnow.

NOEC: 6.25% effluent by volume
LOEC: 12.5% effluent by volume

The effluent did not affect the growth of larval fathead minnow. See Tables A12-1, A12-2, and A12-3 for additional data.

TABLE A12-1. SURVIVAL OF FATHEAD MINNOW LARVAE AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Number Tested	No. Alive at End of Test	Percent Alive
JHU/APL-AES	A	10	9	90
Diluent	B	10	8	80
Water	C	10	10	100
	D	10	9	90
APG	A	10	8	80
Diluent	B	10	7	70
Water	C	10	8	80
	D	10	6	60
6.25	A	10	8	80
	B	10	7	70
	C	10	8	80
	D	10	9	90
12.5	A	10	8	80
	B	10	5	50
	C	10	7	70
	D	10	8	80
25	A	10	6	60
	B	10	7	70
	C	10	5	50
	D	10	8	80
50	A	10	7	70
	B	10	8	80
	C	10	4	40
	D	10	5	50
100	A	10	6	60
	B	10	6	60
	C	10	7	70
	D	10	6	60

Results: The effluent affected the survival of the juveniles. The analysis of the data is summarized on the next page.

Statistical Analysis of Fathead Minnow Larval Survival

Data Transformation:

Arc-sine square-root transformation was used for dealing with values of 0 and 1.0 (Horning and Weber, 1985).

Chi-Square Test for Normality:

Calculated test statistic:	1.88
Alpha value:	0.01
Critical value:	5.30
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	4.22
Alpha value:	0.01
Critical value:	16.81
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	3.82
Alpha value:	0.05
Critical value:	2.57
Conclusion:	Reject the null hypothesis that all groups are equal

Dunnett's Test:

Calculated test statistic:	See Table A12-2
Alpha value:	0.05
Critical value:	2.46
Conclusion:	Reject the null hypothesis that all groups are equal

TABLE A12-2. RESULTS OF DUNNETT'S TEST ON FATHEAD MINNOW LARVAL SURVIVAL AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by volume)	No. of Reps	Percent Survival	T Statistic	Significance
JHU/APL-AES Diluent Water	4	90.0		
APG Diluent Water	4	72.5	2.51	*
6.25	4	80.0	1.53	
12.5	4	70.0	2.78	*
25	4	65.0	3.38	*
50	4	60.0	3.93	*
100	4	62.5	3.71	*

* Significantly different at alpha = 0.05 (Dunnett critical value = 2.46).

TABLE A12-3. GROWTH OF FATHEAD MINNOW LARVAE AFTER 7 DAYS OF EXPOSURE TO APG-WWTP EFFLUENT.

Concentration (% Effluent by Volume)	Rep	Dry Weight (mg)	Mean Dry Weight (mg)
JHU/APL-AES Diluent Water	A	0.52	
	B	0.37	
	C	0.34	
	D	0.43	0.42
APG Diluent Water	A	0.45	
	B	0.39	
	C	0.25	
	D	0.38	0.37
6.25	A	0.45	
	B	0.43	
	C	0.26	
	D	0.30	0.36
12.5	A	0.31	
	B	0.38	
	C	0.46	
	D	0.40	0.39
25	A	0.38	
	B	0.31	
	C	0.28	
	D	0.25	0.31
50	A	0.24	
	B	0.59	
	C	0.35	
	D	0.42	0.40
100	A	0.32	
	B	0.38	
	C	0.33	
	D	0.22	0.31

Results: The effluent did not affect the growth of the juveniles. The analysis of the data is summarized on the next page.

Statistical Analysis of Fathead Minnow Larval Growth

Data Transformation:

None

Chi-Square Test for Normality:

Calculate test statistic:	1.88
Alpha value:	0.01
Critical value:	13.28
Conclusion:	Fail to reject the null hypothesis that the data are normally distributed

Bartlett's Test for Homogeneity of Variances:

Calculated test statistic:	3.83
Alpha value:	0.01
Critical value:	16.81
Conclusion:	Fail to reject the null hypothesis that the variances are homogenous

ANOVA:

Calculated test statistic:	0.88
Alpha value:	0.05
Critical value:	2.57
Conclusion:	Fail to reject the null hypothesis that all groups are equal

DOCUMENT DISTRIBUTION LIST

<u>No. of Copies</u>

No. of Copies	
6	Commander U.S. Army Medical Research and Development Command (USAMRDC) ATTN: SGRD-RMI-S Fort Detrick, Frederick, MD 21702-5012
1	Commander U.S. Army Aberdeen Proving Ground Support Activity Directorate of Safety, Health, and Environment STEAP-SH-EE Aberdeen Proving Ground, MD 21005